Persistence of exogenous organic carbon in soil as a cultivation property

Dissertation

For obtaining the academic degree

Doctor of Philosophy

(Ph. D.)

Handed in at Faculty of Life Sciences, Humboldt-University of Berlin

By

Paul Mewes

President of Humboldt-University of Berlin

Prof. Dr.-Ing. Dr. Sabine Kunst

Dean of Faculty of Life Sciences

Prof. Dr. Bernhard Grimm

Doctoral Degrees Committee

1. Prof. Dr. Christof Engels

2. Prof. Dr. Paolo Nannipieri

3. Prof. Dr. Eckhardt George

Day of the oral exam: 21st July 2017

For **Ilona Rahel**, funding me with love, life, and lodging.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
C	Carbon
C_{org}	Organic carbon concentration in soil
CI	Confidence interval
EOC	Exogenous organic carbon
CEL	Cellulose
CUE	Carbon use efficiency
DM	Dry matter
DOM	Dissolved organic matter
FYM	Farmyard manure
GPP	Gross primary production
HEM	Hemicellulose
IOSDV	International organic nitrogen soil fertilisation history experiment
LIC	Lignin (also referred to as 'lignocellulose')
LTE	Long-term field experiment
MBC	Microbial biomass carbon
N	Nitrogen
N_{min}	Mineral nitrogen
N_t	Total nitrogen concentration
NEP	Net ecosystem production
NECB	Net ecosystem carbon balance
NEE	Net ecosystem exchange
NBP	Net biome production
NDF	Neutral detergent fibre
NON	omitted soil fertilisation history
NPP	Net primary production
pH	potentio Hydrogenii
PI	Prediction interval
C_{pot}	Potential residual organic carbon
SCD	Stepwise chemical digestion
SGM	Straw-green manure
SOC	Soil organic carbon
SOL	Soluble organic matter

Summary

In agriculture, different organic fertilisers and plant-derived sources for exogenous organic carbon (EOC) are applied to soil for compensation of SOC losses which are associated with crop production. When applying organic materials to soil, e.g. plant residues, farm fertilisers, and biochar, it is of special interest, which proportion accounts for SOC formation and is not mineralised within a short period of time. This PhD study focused on the assessment of the proportion accounting for SOC formation for different types of EOC with the main aim to inform farmers, agricultural extension services and policy makers about effects of agronomic management on soil fertility and soil carbon sequestration.

The proportion of EOC, accounting for SOC formation has been investigated in long-term field experiments for a number of typical organic fertilisers and crop species in middle Europe. The evaluation of recently introduced crop species requires for less time-consuming and less expensive methodology. In complementation of previous long-term field experiments, this work is part of a concept, which firstly measures the EOC-input from crop residues and organic fertilisers in field experiments, lasting e. g. 3 years and secondly evaluates the fraction of EOC remaining (C_{pot}) after decomposition in laboratory incubation experiments, measuring EOC-induced CO_2-release from prepared soil columns. In that case, we posed three questions: (i) Is potential residual organic carbon (C_{pot}) robust towards different mathematical models and parameter settings in the incubation experiments? In this study, we observed decomposition of wheat straw, maize stubble, and residues of anaerobic maize digestion for biogas production (digestates) in different soils, different temperatures, and subsequent glucose (C) addition and simulated the course of cumulative carbon loss with different mathematical models to derivate C_{pot}. (ii) Is C_{pot} predictable by the biochemical composition of EOC? In this study, we firstly compared biochemical composition and decomposition of different plant residues (straw, stubbles, coarse roots, fine roots, and litter) from maize, sorghum, Sudan grass, winter cereals, oats, and pea in sole cropping, from maize, sorghum, Sudan grass, and winter cereal in double cropping, and from maize-sorghum and pea-oats in intercropping. Secondly, we extended the scope on different types of EOC, comparing plant residues, organic fertilisers, urban composts, digestates, and biochar. (iii) How does N fertilisation, as applied in different time-scales, affect C_{pot} of plant residues? In this study, we observed straw decomposition in soil samples of the international nitrogen long-term fertilisation experiment (IOSDV) in the presence and absence of mineral N-supply in the short-term. We hypothesized that (ii) differences in the fraction of EOC maintaining for SOC are due to differences in biochemical composition but also depend on (iii) long-term fertilisation practice of the soil and (i) specific environmental conditions in the incubation experiment.

The biochemical composition of EOC was characterised by the elemental concentration of C and N, the concentration of water-soluble carbohydrates (WSC) and the proportions of hemicelluloses (HEM), cellulose (CEL) and lignin (LIC) in g per kg dry matter. In the incubation experiments, EOC was

homogeneously incorporated into soil and EOC-induced CO_2-release was measured for 210 – 310 d in closed vessels at 22 °C and optimal moisture (50 % of max. water-holding capacity).

Despite the long incubation duration, total C release could not account for initial C application and mathematical models, considering a potentially degradable C fraction simulated the course of cumulative C loss best. We found potential residual organic carbon (C_{pot}) to be majorly influenced by the biochemical composition of EOC and by the incubation temperature, whereas mathematical model, soil type, glucose (C) and mineral N additions were of minor influence. We observed a progressive decrease of the rate of maize-stubble-induced CO_2-release due to cooling from 22 °C to 6 °C, which largely increased C_{pot}. However, this increase of C_{pot} was not robust towards subsequent rewarming to 22 °C, as microbial activity recovered and maize stubbles were decomposed to an equal stage like stubbles incubated at 22 °C since the beginning of incubation. Potential residual organic carbon largely differed between different types of plant residues, different crop species, and for some crop species between different cropping systems and the cultivation year. The most carbon remained from fine roots, lowest from stubbles and coarse roots. Differences between crop species were apparent for stubbles, coarse roots, and fine roots, not for litter. Potential residual organic carbon was higher for pea than for winter cereal, but it was lowest for maize, sorghum and Sudan grass (C4-plants). The stubbles and coarse roots of the C4-plants contained extraordinarily high concentrations of water-soluble carbohydrates, especially when they were cultivated as second crop after green cut winter-cereal. Potential residual organic carbon of plant residues in double cropping systems was therefore lower than in sole cropping. Decomposition and C_{pot} of plant residues from intercrops differed from that expected from sole crops of the species which are included in the intercrops. Urban compost, organic fertilisers, digestates, and biochar induced less CO_2-release than plant residues. Urban composts and biochar virtually remained completely in soil, while organic fertilisers and digestates induced more CO_2-release depending on the original substrate. Hydrothermal char, which was pyrolysed at low pressure and low temperature contained large amounts of water-soluble carbohydrates, inducing high CO_2-release. Organic fertilisers, urban composts, and digestates partially constituted microbial products and had low C:N-ratios. Nitrogen fertilisation in the long-term and mineral N supply in the short-term prevented N-limitation of microbial decomposition of plant residues. Nitrogen increased microbial growth and decomposition of plant residues initially, and led to higher C_{pot}. Mineral N supply increased N-immobilisation during decomposition and led to higher soil-pH.

In conclusion, we verified the hypotheses (i) - (iii) and propose the biochemical prediction of C_{pot} in g C (kg EOC)$^{-1}$ for plant residues as $I_{pot} = 269 + 13\ N - 0.5\ WSC + 0.7\ CEL + 1.5\ LIC$, ($R^2 = 0.84$, $n = 40$) and different types of EOC as $I_{pot} = 924 - 1.9\ C + 2.0\ LIC$, ($R^2 = 0.92$, $n = 30$). The values need to be confirmed by litterbag experiments in a cultivated field. The large influence of biochemical composition and the influence of long-term N-fertilisation on initial decomposition of EOC provided evidence for persistence of EOC to be a cultivation property, assessable as integral part of agricultural management.

Zusammenfassung

Der Anbau von landwirtschaftlichen und gärtnerischen Nutzpflanzen ist im Allgemeinen mit Humusverlusten verbunden. Diese Humusverluste können durch Zufuhr von organischen Düngern und verschiedene pflanzliche Quellen für organischen Kohlenstoff (C), wie z.B. ober- und unterirdische Ernterückstände, ausgeglichen werden. Exogener organischer Kohlenstoff (EOC), der dem Boden zugeführt wird, induziert CO_2-Freisetzung während des Abbaus, Umbaus und der Humifizierung. Der Anteil des EOC, der nicht innerhalb einer kurzen Zeitspanne wieder als CO_2 in die Atmosphäre freigesetzt wird sondern potenziell im Boden verbleibt, kann anbaubedingte Humusverluste ausgleichen und steht somit in besonderem landwirtschaftlichen Interesse. Diese Doktorarbeit versucht den Anteil verschiedener EOC Quellen, der potenziell im Boden verbleibt und anbaubedingte Humusverluste ausgleichen kann, experimentell in Laborversuchen unter standardisierten Umweltbedingen zu ermitteln, mit dem Ziel Landwirte, landwirtschaftliche Beratungsdienste und die Agrarpolitik zu informieren.

Bisher wurde in jahrzehntelangen Freilandversuchen für einige organische Dünger und Kulturarten der Anteil des organischen Kohlenstoffs ermittelt, der in den Bodenhumus übergeht. Freilandversuche sind zeit- und kostenintensiv, weswegen sie sich kaum für die Bewertung neuer Kulturarten eignen. In Ergänzung zu den Freilandversuchen ist diese Arbeit Teil eines neuen Konzeptes für die landwirtschaftliche Humusbilanzierung, in dem über einen kürzeren Versuchszeitraum (3 Jahre) zum einen die C-Einträge durch Pflanzenreste und organische Dünger in den Boden gemessen werden und zum anderen der Abbau der C-Eintragsquellen (EOC) in Inkubationsversuchen verglichen wird, in denen die EOC-induzierte CO_2-Freisetzung aus präparierten Bodensäulen gemessen werden kann. Vor diesem Hintergrund wurden 3 Versuchsfragen an die Ermittlung des potentiell verbleibenden Kohlenstoffs verschiedener EOC Quellen (C_{pot}) im Laborexperiment gestellt: (i) Ist C_{pot} robust gegenüber verschiedenen mathematischen Modellansätzen und Parametereinstellungen im Inkubationsversuch? In dieser Studie wurde der Abbau von Weizenstroh, Maisstoppeln und Rückständen aus Maisvergärung in verschiedenen Böden, bei unterschiedlichen Temperaturen und einer nachträglichen C-Zugabe untersucht und die ermittelte Abbaukurve mit verschiedenen mathematischen Modellen simuliert um C_{pot} abzuleiten. (ii) Kann C_{pot} durch die biochemische Zusammensetzung des EOC vorhergesagt werden? In dieser Studie wurde zunächst das Abbauverhalten und die biochemische Zusammensetzung verschiedener Pflanzenrückstände (Restpflanze / Stroh, Stoppeln, Grobwurzeln, Feinwurzeln, natürlicher Bestandsabfall) von Mais, Sorghum, Sudangras, Wintergetreide, Hafer, Erbse im Einkultursystem, von Mais, Sorghum, Sudangras und Wintergetreide im Zweikultursystem und von Mais-Sorghum und Erbse-Hafer im Mischkultursystem verglichen. In einem zweiten Schritt wurden Pflanzenrückständen im Allgemeinen mit organischen Düngern, Komposten, Rückständen aus anaerober Vergärung in der Biogasproduktion (Gärrückstände) und Biokohlen im Boden verglichen. (iii) Welchen Einfluss hat die kurz- und langfristige Stickstoffdüngung auf C_{pot}? In dieser Studie wurde

der Strohabbau in verschiedenen Böden des Internationalen Organischen Stickstoffdauerdüngungsversuches (IOSDV) mit und ohne mineralischer N-Zugabe verglichen. Wir stellten die Hypothesen auf, dass (i) C_{pot} verschiedener pflanzlicher C-Quellen von der Inkubationstemperatur, dem Boden, der Kohlenstoff- und (iii) Stickstoffverfügbarkeit für Mikroorganismen abhängen, (ii) unterschiedliche C_{pot} verschiedener pflanzlicher C-Quellen aber auf deren unterschiedlichen biochemischen Zusammensetzungen beruhen.

Die biochemische Zusammensetzung der EOC-Quellen wurde durch die gravimetrische Elementarkonzentration von Kohlenstoff (C) und Stickstoff (N), den Gehalt wasserlöslicher Kohlehydrate (WSC), und die Anteile an Hemizellulose (HEM), Zellulose (CEL) und Lignin (LIC) in g pro kg Trockenmasse dargestellt. In den Inkubationsversuchen wurden die EOC-Quellen gleichmäßig mit Boden vermischt und über 210-310 Tage die CO_2-Freisetzung in geschlossenen Glasgefäßen bei 22°C und optimaler Feuchte (50 % der maximalen Wasserhaltekapazität des Bodens) gemessen.

Trotz der langen Inkubationsdauer entsprach die EOC-induzierte CO_2-Freisetzung in keinem Fall der zugeführten C-Menge und mathematische Modellansätze, die von einem potentiell abbaubaren Kohlenstoffanteil ausgingen simulierten die Abbaukurve am besten. Den potenziell verbleibenden Kohlenstoff (C_{pot}) fanden wir in hohem Maße beeinflusst durch die biochemische Zusammensetzung von EOC und der Inkubationstemperatur, wohingegen die Wahl des mathematischen Modells, der Versuchsboden, Glukose- (C) und mineralische Stickstoffzugaben (N) einen geringfügigeren Einfluss auf C_{pot} ausübten. Wir beobachteten eine Temperatur-bedingte Erhöhung von C_{pot} um ein Vielfaches für Maisstoppeln bei Temperatursenkung von 22 °C (Standardtemperatur) auf 6 °C, die jedoch durch eine anschließende Temperaturerhöhung aufgehoben werden konnte. Der potenziell im Boden verbleibende Kohlenstoff unterschied sich stark zwischen den verschiedenen Pflanzenrückständen und wurde von der Kulturart, für einzelne Kulturarten von der Stellung im Anbausystem und dem Anbaujahr beeinflusst. Am meisten Kohlenstoff verblieb von Feinwurzeln, am wenigsten von Stoppeln und Grobwurzeln im Boden. Kulturartspezifische Unterschiede bestanden in Stoppeln, Grob- und Feinwurzeln, nicht aber im Bestandsabfall. Der Anteil des potenziell im Boden verbleibenden Kohlenstoffs war höher für die Ernterückstände der Erbse als für die des Wintergetreides; am geringsten war er für die Ernterückstände von Mais, Sorghum und Sudangras (C4-Pflanzen). Die Stoppeln und Grobwurzeln der C4-Pflanzen enthielten außerordentlich große Mengen wasserlöslicher Kohlehydrate, insbesondere beim Anbau in Zweitfruchtstellung im Zweikultursystem mit Grünschnittgetreide. Der Anteil des potenziell im Boden verbleibenden Kohlenstoffs der Ernterückstände des Zweikultursystems war somit geringer als in Einkultursystemen. Darüber hinaus unterschied sich der Abbau von Ernterückständen in Mischkultursystemen von dem erwarteten Abbau aus Einkultursystemen der Kulturen, die im Mischkultursystem angebaut wurden. Komposte, Wirtschaftsdünger, Gärsubstrate und Biokohlen induzierten geringere CO_2-Freisetzungen als pflanzliche Kohlenstoffquellen. Der Kohlenstoff von Komposten und Biokohlen verblieb im Abbauversuch nahezu vollständig im Boden, während

Wirtschaftsdünger und Gärrückstände in Abhängigkeit vom Ausgangssubstrat unterschiedlich starke CO_2-Freisetzungen induzierten. Hydrothermalkohlen, die bei geringem Druck und niedriger Temperatur entstanden enthielten zu einem großen Anteil wasserlösliche Kohlenhydrate und induzierten ebenfalls starke CO_2-Freisetzungen. Wirtschaftsdünger, Gärrückstände und Komposte sind zum Teil Produkte mikrobiellen Abbaus und hatten somit ein enges C:N-Masseverhältnis. Die langfristige Stickstoffdüngung und kurzfristige mineralische Stickstoffzugaben zu pflanzlichen Kohlenstoffquellen konnten Stickstoffmangelsituationen im mikrobiellen Abbau vorbeugen. Sie erhöhten anfänglich das mikrobielle Wachstum, beschleunigten den Abbau von Stroh und führten zu einem höheren Kohlenstoffanteil, der potenziell im Boden verbleibt. Kurzfristige N-Zugaben erhöhten die N-Immobilisierung während des Abbauprozesses und den Boden-pH.

Wir konnten die Hypothesen (i)-(iii) annehmen und Schätzgleichungen für C_{pot} in g C (kg EOC)$^{-1}$ pflanzlicher C-Quellen $I_{pot} = 269 + 13\ N - 0.5\ WSC + 0.7\ CEL + 1.5\ LIC$, ($R^2 = 0.84$, $n = 40$), sowie für C_{pot} verschiedener EOC Quellen $I_{pot} = 924 - 1.9\ C + 2.0\ LIC$, ($R^2 = 0.92$, $n = 30$) nach den untersuchten biochemischen Parametern ableiten. Die Werte müssen noch durch nachfolgende Streubeutelversuche im Freiland bestätigt werden. Der starke Einfluss der biochemischen Zusammensetzung sowie der Einfluss der langfristigen N-Düngung auf den kurzfristigen Abbau, führen uns zu dem Schluss, dass die Persistenz (Verweilzeit, Umsatzzeit) von organischen Düngern und verschiedenen pflanzliche Quellen für organischen Kohlenstoff (C) eine kultursystemspezifische Eigenschaft ist und zu einem großen Teil durch landwirtschaftliches Management gesteuert werden kann.

1 Introduction

1.1 Terrestrial carbon cycle

The terrestrial carbon (C) cycle is characterised by the two basic, antagonistic processes: C assimilation (photosynthesis) and C dissimilation (respiration) (Campbell et al., 1999). Plants, as primary producers, fix atmospheric CO_2 by photosynthesis which is further incorporated into biomass. The amount of annually synthesised organic carbon, identified as gross primary production (GPP), thereby forms the CO_2-sink in terrestrial ecosystem balancing (Woodwell and Whittaker, 1968). Approximately half of photosynthetically fixed carbon is lost by photorespiration and the plant's autotrophic respiration. The difference between GPP and autotrophic respiration of plants is defined as net primary production (NPP). Net primary production is represented by the whole plant biomass, above and below ground. Aside from plants, intact ecosystems harbour animals and microorganisms either, which release CO_2 by so called heterotrophic respiration. Whereas the portion of released CO_2 in heterotrophic respiration derived from animals is minor, the vast majority of released CO_2 is caused by microorganisms living in soil and decomposing organic materials. In short, heterotrophic respiration refers to the carbon lost by organisms in ecosystems other than the plants. The sum of auto- and heterotrophic respiration is ecosystem respiration. Taking these core carbon fluxes into account, the difference between GPP and ecosystem respiration is defined as net ecosystem production (NEP). Net ecosystem production was considered to represent the carbon accumulation or loss in undisturbed ecosystems (Woodwell and Whittaker, 1968), but actually deviates because of carbon exchange with neighbouring ecosystems (Fisher and Likens, 1973, Lovett et al., 2006). A new concept, called net ecosystem carbon balance (NECB), does not consider just C fixation and respiration which results in net ecosystem exchange (NEE). NECB considers all carbon exchange fluxes, e.g. when inorganic or organic C enters or leaves in dissolved form, leaching loss or lateral transfer of particulate C from the ecosystem, the emission of volatile organic C (VOC), methane, and carbon monoxide, by processes such as soot and CO_2 emission during fires, water and wind deposition and erosion, and anthropogenic transport or harvest. (Chapin et al., 2006, Luyssaert et al., 2007). Net ecosystem carbon balance (NECB) precisely expresses carbon accumulation (or loss) in an ecosystem. Over large temporal and spatial scales, NECB results in net biome production (NBP), expressing carbon sequestration from the atmosphere (Chapin et al., 2006, Luyssaert et al., 2007). The persistence of organic carbon thereby depends on the sequestering ecosystem component and even differs for components of NPP (Luyssaert et al., 2007): Carbon in foliage and fine root tissues has mean transit times of days to months, whereas carbon in woody tissues resides for years to decades. It was calculated, that terrestrial ecosystems accumulate 2-4 Gt C per year (Schimel et al., 2001). Luyssaert et al. (2008) identified old-growth forests (between 15 and 800 years of age) as a global carbon dioxide sink, sequestering at least 1.3 ± 0.5 Gt C per year.

1.2 Organic carbon cycling in soil

Plant biomass, e.g. litter and exudates are decomposed and transformed by soil organisms to form SOC, which might be further mineralised by microbial activity or stabilized. Soil organic carbon (SOC) pool size is dependent on the balance between formation of SOC from decomposition of plant litter and its mineralisation to inorganic carbon. Persistence of SOC may range from decades to centuries (Amelung et al., 2008). Today is known, that SOC is the largest terrestrial carbon pool (Schlesinger and Bernhardt, 2013), at least three times as much carbon is stored as is found in either the atmosphere or in living plants (Fischlin et al., 2007). In the background of global warming and SOC being a potential CO_2 sink, it is important to get further insight into SOC cycling and determinants of persistence. Contrary to early concepts of recalcitrance, molecular structure has influence on decomposition, but does not control long-term decomposition of soil organic matter (Schmidt et al., 2011). For long-term decomposition, environmental stabilisation mechanisms, such as physical disconnection from enzymes, decomposers, and electron-acceptors or sorption and desorption to the soil solid phase regulate the probability of decomposition much stronger, characterising persistence of soil organic matter as an ecosystem property (Schmidt et al., 2011). Smith et al. (1997) assume that stable SOC is formed primarily from recalcitrant plant litter. However, even labile components of plant litter may form mineral-stabilized soil organic matter as well (Cotrufo et al. 2013).

Aside from plant biomass, microbial metabolic products complement SOC (Kogel-Knabner, 2002). Responses of microbial carbon use efficiency (CUE) on environmental changes, such as temperature and nitrogen availability, therefore substantially influences SOC formation (Manzoni et al., 2012b).

1.3 Decomposers and stable organic matter formation (humification)

The application of plant residues provides both energy-rich water-soluble carbon compounds and structural carbon compounds of a lower energetic level to the soil microbial community (Cotrufo et al., 2015). Microbial decomposition of newly applied plant residues can be measured as progressive loss of chemical components or release of CO_2, while the formation of SOC occurs via biochemical and physical transfer pathways, which can be identified through isotopic tracing (Cotrufo et al., 2015). On the biochemical pathway, water-soluble carbon compounds are high efficiently incorporated into microorganisms or transformed into microbial products, whereas structural carbon compounds of plant residues are being decomposed more slowly than water-soluble carbon compounds (Cotrufo et al., 2013). Water-soluble carbon compounds thereby contribute to the high persistent mineral-associated SOC fraction, while structural components form the less persistent coarse particulate SOC fraction (Cotrufo et al., 2015). Despite this heterogeneity in persistence, both fractions constitute well decomposed organic C in soil as indicated as humus by the SSSA (2008).

Microbial decomposition of plant residues and other types of EOC, occurs over years in cultivation depending on a variety of environmental conditions (Wessen and Berg, 1986), but can be observed within months in laboratory incubation (Sleutel et al., 2005). Incubation experiments enable the

measurement of EOC-induced CO_2-release from prepared soil columns, in which plant residues and other types of EOC are homogeneously incorporated under controlled environmental conditions. Although SOC and likewise EOC are completely decomposable by the soil microbial community irrespective of elemental composition, aromaticity, and molecular-size of carbon compounds (Schmidt et al., 2011), the EOC-induced CO_2-release does not completely account for the initially applied EOC, leaving a residual organic carbon fraction (C_{pot}) in incubated soil columns (Lashermes et al., 2009).

Nitrogen availability for microorganism plays an important role for the incorporation of carbon compounds into microorganisms and the formation of stable decomposer products, as the carbon to nitrogen ratio is higher for plant residues than for microbial biomass (Kirkby et al., 2011). The addition of mineral N in incubation experiments therefore substantially increases the formation of mineral-associated SOC, as indicated as increased humification efficiency (Kirkby et al., 2013, 2014).

1.4 Carbon cycling and balancing in agro-ecosystems

SOC balancing is of special agronomic interest, as humus mediates several soil functions, offering habitats for microorganisms, capturing and releasing nutrients, storing water, and enabling soil structure, which all serve for soil fertility (Scheffer et al., 1979). The agronomical approach therefore aims to sustain the soil organic matter level, that site specifically enables high yield levels by balancing demand and supply of organic matter (OM) in cultivation (Brock et al., 2013). Cultivation encompasses the annual growth of arable crops, allowing the determination of various NPP components (Crop yield, stubble, coarse root, fine roots, litter and fertiliser, Figure 1). Crop residues are supplied to SOC cycling, while crop yields are exported to livestock farming and food industry. Farm fertilisers are applied in return to maintain soil organic carbon (SOC) pools. Since a huge portion of the carbon applied with EOC is released as CO_2 by microbial respiration during decomposition, it is of special interest to be able to estimate the amount of carbon, which is not released by decomposition in a short period of time (e. g. one year), but somehow stabilized for longer periods of time, contributing to maintain SOC and thereby humus status of the soil. In Germany, the standard humus-balance method can be used. This method evaluates the contribution of EOC to SOC replacement on behalf of temporal changes of SOC stocks (Ebertseder et al., 2014). The fraction of EOC, which is converted to more resistant SOC is defined as humification coefficient (Hénin and Dupuis, 1945). This coefficient is used to simulate the formation of SOC in the introductory carbon balance model (ICBM) family (Andren and Katterer, 1997). Nevertheless, determining carbon persistence in agro-ecosystems is difficult since measuring gaseous ecosystem carbon fluxes (NEE) and dissolved carbon exchanges is extensive and due to annual changes ineffective to quantify for the spatial scale of agricultural fields. Net ecosystem production (NEP) could balance the core carbon flux for carbon cycling in agro-ecosystems as difference of NPP and heterotrophic respiration (Luyssaert et al., 2007). Carbon balancing in cultivation therefore requires an integrated framework, determining and measuring components of NPP and identifying stages of decomposition according to carbon persistence.

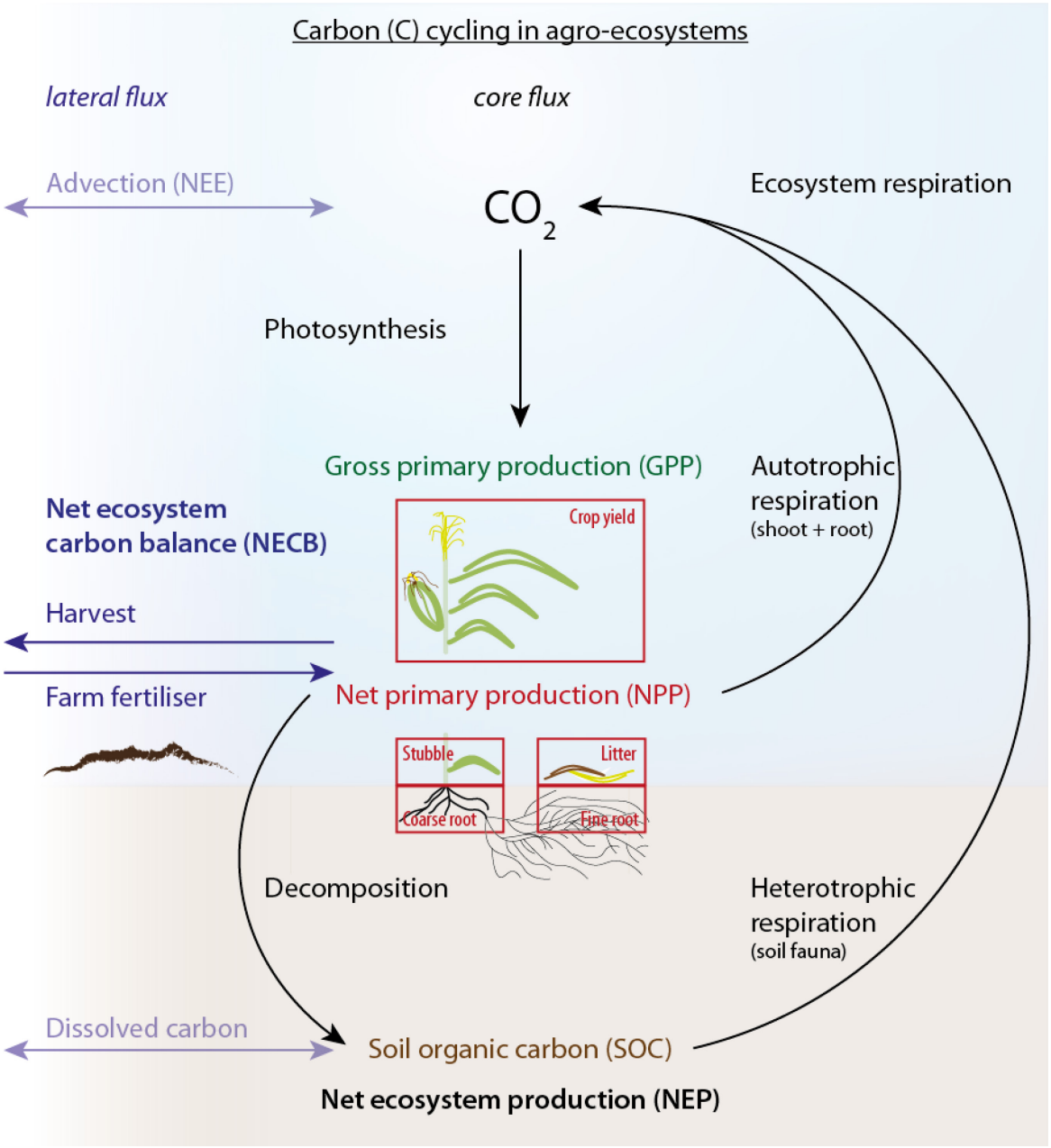

Figure 1 A synopsis of terrestrial carbon cycling in agro-ecosystems and balancing concepts: Crops fix CO_2 by photosynthesis (gross primary production; GPP) and emit CO_2 due to autotrophic respiration. Net primary production (NPP), defined as difference of both, is represented by the biomass produced by the crop plant (red). Fixed CO_2 is found in crop yield, crop residues (stubble, coarse roots, litter, and fine roots) and root exudates. Carbon-exchange with neighbouring ecosystems occurs when harvested crop yield is exported for animal and human nutrition and fertilisers return. Together with crop residues, farm fertilisers are incorporated into topsoil. Soil organisms steadily mineralise organic material in soil (heterotrophic respiration) and evolving CO_2 returns to the atmosphere. Net Ecosystem Production (NEP) refer to NPP minus EOC-induced CO_2-release in heterotrophic respiration (black bold). NEP often deviates from actual carbon accumulation. The net rate of carbon accumulation in an ecosystem is illustrated by the concept of net ecosystem carbon balance (blue), based on net ecosystem exchange via advection and further lateral carbon fluxes.

1.5 Objective: SOC formation – Impact of different environmental conditions and agricultural determinants of EOC persistence

SOC can be divided into living biomass and dead organic substance (humus). When adding organic material to soils, it is of special interest, which proportion accounts for SOC formation and is not mineralised within a short period, e.g. a year. This can be analysed by decomposition studies.

In agriculture, EOC is applied to soil for compensation of SOC losses which are associated with crop production. In soil, EOC is in part used for heterotrophic respiration and in part transferred into the SOC pool, in a process called "humification". The amount of EOC needed to compensate for SOC losses is dependent on the amount of cropping-induced SOC losses and the efficiency of humification. The focus of this PhD study was on assessing the efficiency of humification of different types of EOC (with the main aim to inform farmers, agricultural extension services and policy makers about effects of agronomic management on soil fertility and soil carbon sequestration).

Traditionally, effects of EOC application on SOC content are investigated in long-term field experiments. However, due to the large costs associated with long-term field experiments, only few types of EOC were tested so far, e.g., sewage sludge and farmyard manure (Katterer et al., 2014). For the assessment of differences among types of EOC in their effects on SOC content, incubation studies under controlled conditions have been used (Jensen et al., 2005). In these studies humification coefficients may be derived from quantification of the CO_2 release from soil which is induced by amendment of different types of EOC. In the first part of the thesis (chapter 2), experiments are presented which were focused mainly on methodological aspects of incubation experiments, whereas the focus of the experiments described in the second part of the thesis was on investigation of effects of biochemical composition of EOC (chapter 3 and 4), and of EOC-induced soil conditions (chapter 5) on humification.

Focus of chapter 2 was on identification of a mathematical model which describes the time response of EOC-induced CO_2 release from soil which we measured in incubation studies. To test the robustness of the model towards different soil properties and EOC properties, the EOC-induced CO_2 release was measured in four soils differing in texture, and using two types of EOC with large differences in decomposition (straw, residues from anaerobic digestion of maize for biogas production), and in one of these soils using either different types of plant residues or different types of EOC. The suitability of six different models for simulating our data on CO_2-release was tested. Focus of chapter two was further on alterations of EOC-induced CO_2-release and C_{pot} due to different temperatures during the incubation. Two different temperatures, 22 °C and 6 °C, were initially set for incubation of maize stubble and finally reset to 22 °C after 161 days of incubation. To characterise the temperature dependence of EOC-induced CO_2-release and C_{pot}, the Q_{10}-value was calculated during the initial 161 days of incubation. The recovery of decomposition after resetting from 6 °C to 22 °C was used to test the robustness of C_{pot} towards time-dependent temperature variation. Finally, chapter 2 focused on the impact of the EOC-induced CO_2-release due to the SOC priming effect and a limitation of easily-available carbon

compounds for microorganisms. To identify the SOC priming effect in the EOC-induced CO_2-release, wheat plants had been labelled with the stable carbon isotope ^{13}C during cultivation in the greenhouse. The $^{13}C/^{12}C$-isotopic ratio from wheat shoot biomass was traced in the wheat-shoot-induced CO_2-release during an incubation experiment. To test the limitation of easily-available carbon compounds for microorganisms, glucose was added to one set of soil columns after 35 days of incubation and effects on the EOC-induced CO_2-release were described for wheat-shoot biomass and maize digestate. Our main hypothesis was, that C_{pot} robustly informs about the fraction of EOC, remaining and maintaining SOC status of the soil.

Focus of chapter 3 was on the identification of the fraction contributing to SOC and thereby humus status of the soil for several types of plant residues from different crop species, which emerge importance for energy-crop cultivation (Figure 2). To compare decomposition and C_{pot} of straw, stubbles, litter, coarse- and fine roots from different crop species and in different cropping systems, plant residues were sampled from a field-experiment for an incubation under controlled environmental conditions.

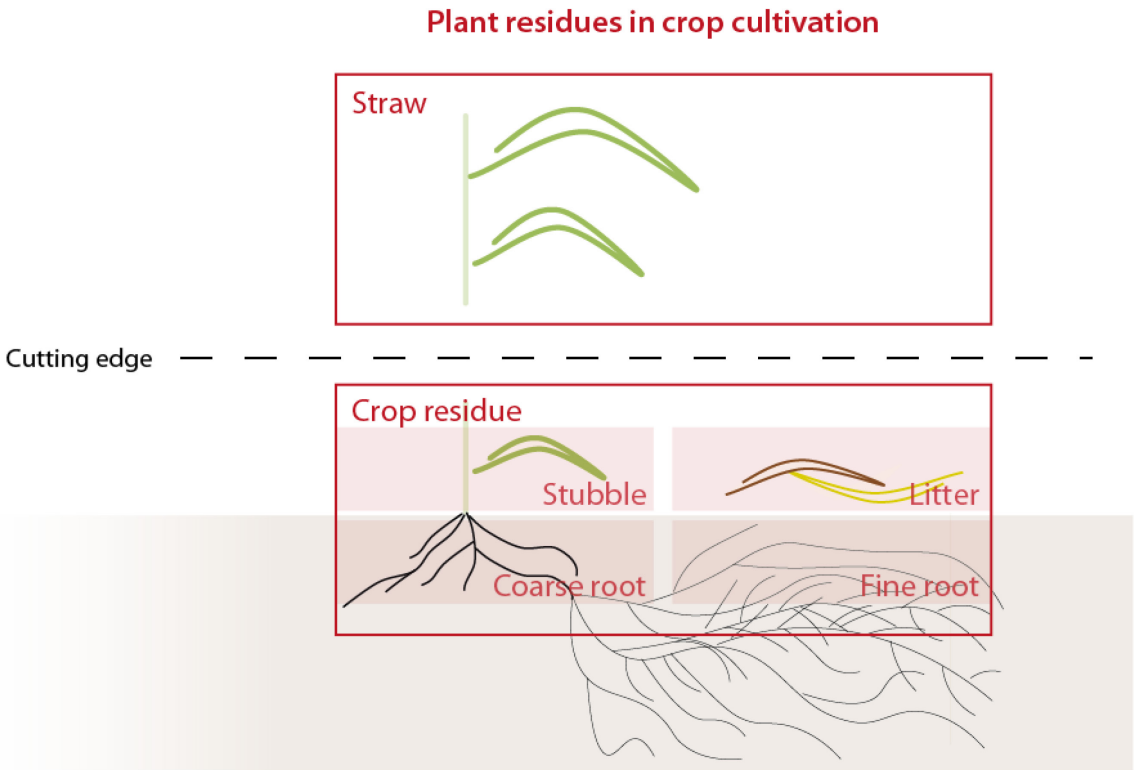

Figure 2 In cropping systems for food and energy production a variety of plant residues (red) at different stages of maturity or senescence, in detail straw and the crop residue (litter, stubble, coarse root, fine root, and rhizodeposition), are annually being incorporated into the soil.

To characterise the influence of the biochemical composition of plant residues on C_{pot}, a fractionation of structural cell-wall constituents and the amount of water-soluble carbohydrates in the organic matter of plant residues were tested as independent variables in regressions to predict C_{pot}. Our main hypothesis was that decomposition of plant residues can be predicted by their chemical composition which, in turn,

is influenced by crop species, residue type, and crop management (plant developmental stage at harvest, sole vs. intercropping, sole crop vs. second crop).

Chapter 4 set focus of chapter 3 into a more general context, as plant residues and other groups of EOC were regarded, containing different samples of straw, digestates, farm fertilisers, urban composts, and biochar from different source areas, which spread over Germany and Switzerland. (Figure 3). Our main hypothesis was that if the applied biochemical parameters were insensitive for biochemical alterations of EOC due to thermal (in case of biochars) or microbial conversion (in case of digestates, farm fertilisers and urban composts), C_{pot} would not be predictable by a common biochemical indicator for all groups of EOC.

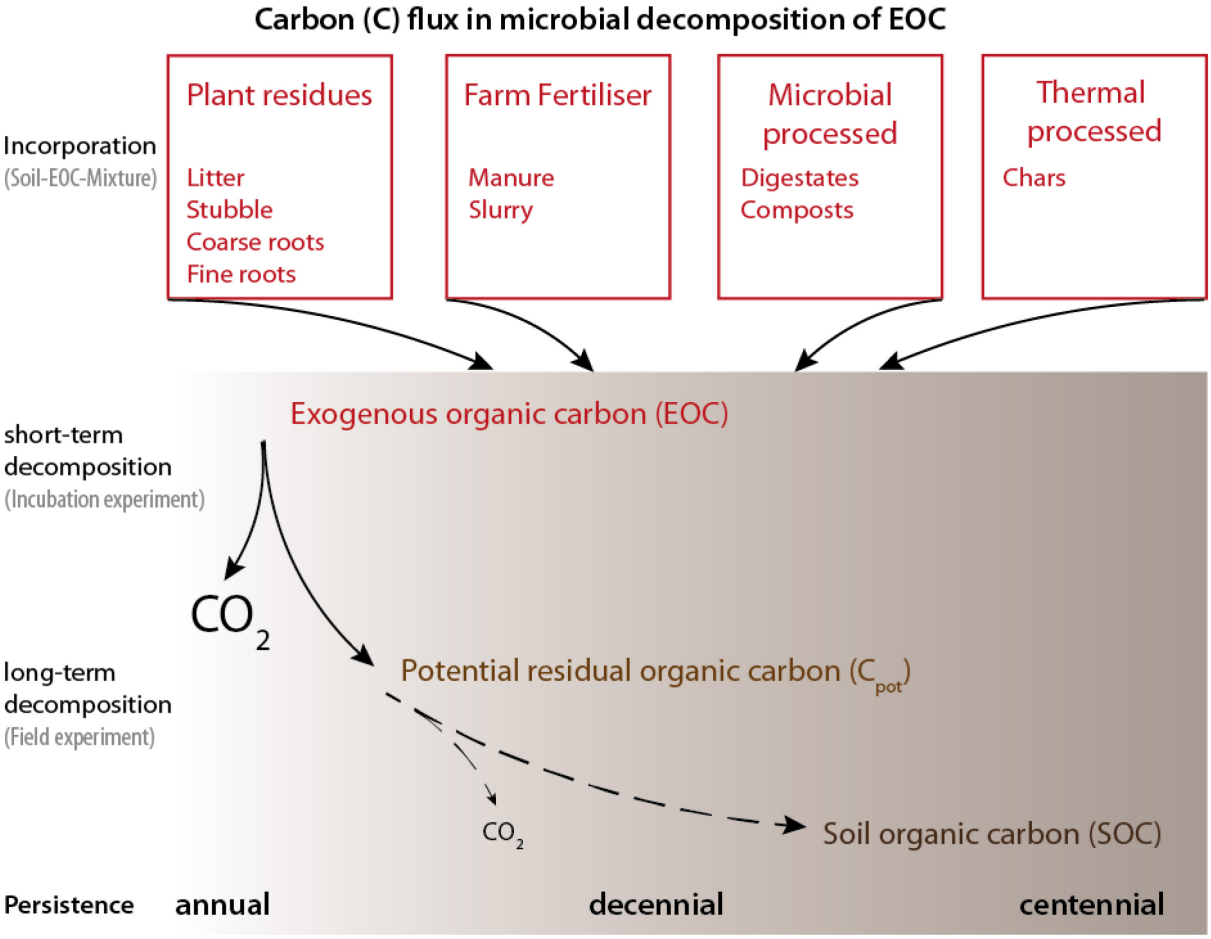

Figure 3 EOC types analysed in this study for their potential contribution to maintain SOC in agro-ecosytems: In crop cultivation different groups of EOC (farm fertilizers, bacterial processed material, plant residues or thermal processed material) can be applied to soil. The sum of contained carbon can be divided into short-term decomposable carbon, persisting for about one year in soil and potentially residual organic carbon (C_{pot}), persisting for decades and contributing to stable soil organic carbon (SOC), persisting for centuries in soil.

Focus of chapter 5 was on the influence of both the N fertilisation history of a given soil and the mineral N supply to a given soil on microbial decomposition of straw, as an aspect of agricultural management (Figure 4). To characterise microbial decomposition of straw, the straw-induced CO_2-release was

measured in an incubation experiment lasting 210 days, microbial biomass carbon and microbial biomass nitrogen were measured after 3 days of incubation, and the mineral N concentration in the soil and pH-value of the soil solution were measured in the beginning and the end of incubation. The N fertilisation history was represented by soil samples from the IOSDV experiment in Berlin-Dahlem, which were fertilised with either no organic amendments, straw-green manure amendments, or farm-yard manure amendments in combination with different levels of mineral N amendments for a duration of 30 years. To differentiate between N fertilisation history and short-term N fertilisation, two sets of sampled soils were prepared - with and without mineral N supply before incubation. Our main hypothesis was, that both mineral N supply before incubation and N fertilisation for a long period of time increase microbial growth, microbial carbon use efficiency, initial decomposition, and C_{pot} of straw.

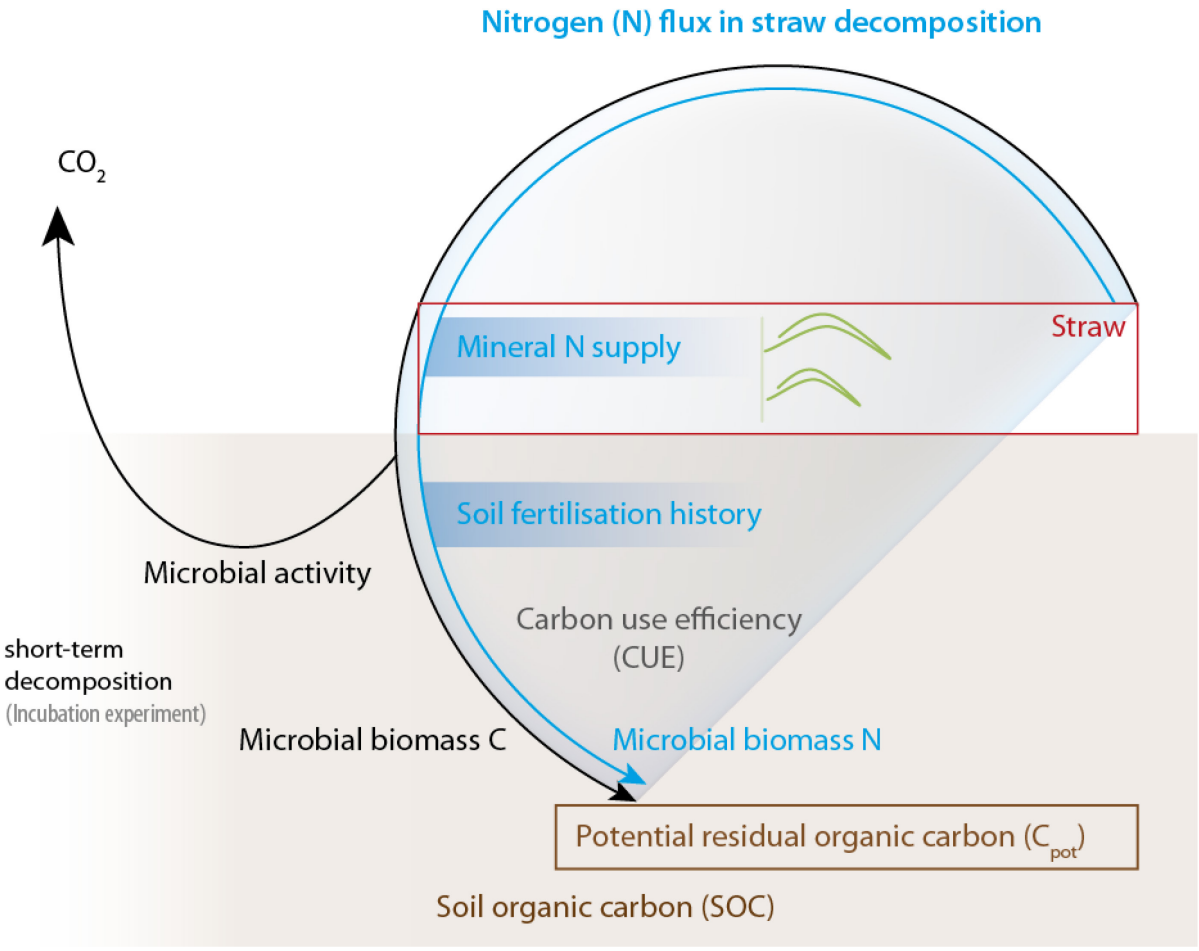

Figure 4 Nitrogen fluxes parallel to EOC decomposition affect microbial activity and microbial growth (black). In cultivation, nitrogen is being fertilised in the long-term or supplied in laboratory incubation (blue). Nitrogen is suspected to enhance microbial carbon use efficiency (grey) and thereby to increase the SOC formation in agro-ecosystems (brown).

2 Decomposition of EOC in the controlled environment of incubation experiments: Simulation, Interpretation, and Environmental parameter setting

2.1 Introduction

2.1.1 Simulation of decomposition by different mathematical models

Incubation experiments homogenise the incorporation of substrates for microbial decomposition into soil (exogenous organic carbon EOC) by a defined rate of EOC addition (incubation ratio) and measure the effect on microbial activity (as EOC-induced CO_2-release) under standardised environmental conditions. The measured course of EOC-induced CO_2-release can be explained by several models, which explicitly describe the process for one or more discrete carbon compartments at constant decay rates, or lumped models, which describe decomposition at time-dependent decay rates, or models, which assume a quality-dependent distribution of decay-rates in the EOC sample (Manzoni et al., 2012a). These models either suppose a complete decomposition of EOC (EOC-induced CO_2-release equals EOC addition) or consider the EOC induced carbon release to be different from EOC addition. If EOC-induced CO_2-release is assumed to be different from initial carbon addition, the mathematical models estimate a potentially biodegradable carbon pool for incubation (Figure 5). Models, which describe decomposition of discrete carbon compartments at constant decay rates by first- or second-order kinetics consider the EOC-induced carbon release to be different from EOC addition. These models were frequently used for the estimation of a stable carbon fraction, which remains in soil at a given incubation duration (Sleutel et al., 2005). Lashermes et al. (2009) estimate potential residual organic carbon in soil (C_{pot}) as the difference between the cumulative EOC-induced carbon release and added EOC at an incubation duration, when the EOC-induced carbon release occurs at the same rate as SOC mineralisation in field experiments, using different models of first- and second-order kinetics. (Sleutel et al., 2005) proposed the second-order kinetic as the most reliable one for C_{pot} - estimations, as it remained relatively robust towards different incubation durations, whereas (Lashermes et al., 2009) rather prolonged incubation duration and preferred a model of two carbon compartments, both released according first-order kinetics.

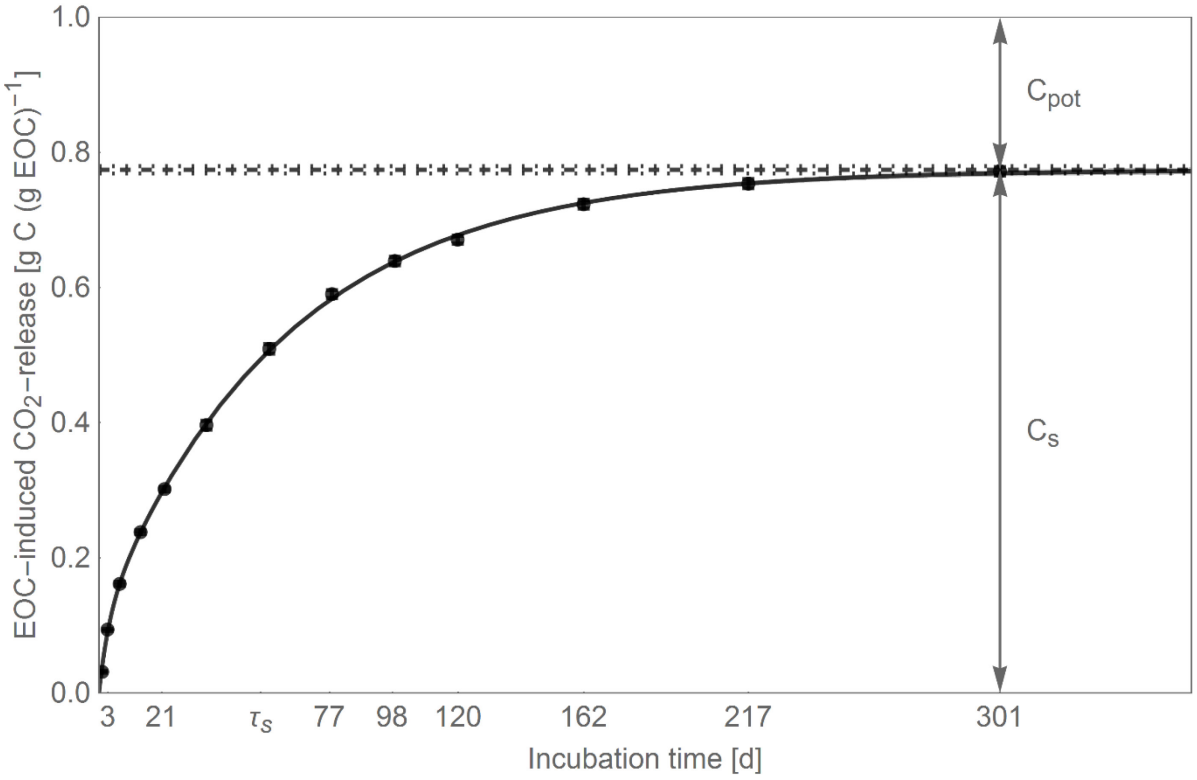

Figure 5 Conceptual framework of EOC decomposition: Despite assuming decomposition of exogenous organic carbon to appear completely, dividing up the process into a short-term decomposition, consecutive in long-term decomposition better fitted observation. C_{pot} as complement to the biodegradable carbon pool C_s is assumed to be solely biodegradable in the long-term under cultivation-specific environmental conditions. Black symbols: Decomposition of wheat straw in soil (Berlin Dahlem) given as mean ± standard error (n = 5), D2 model ± 95 %-CI (shaded), and model limit (dashed) ± 95 %-CI (dotted).

In this study we addressed the questions: (i) Which of the three discrete-compartment-models (D1-D3) describes the course of EOC-induced CO2-release best and delivers the most reliable estimation of C_{pot}? (ii) Do the three discrete-compartment-models (D1-D3) perform the course of EOC-induced CO_2-release better for the special case that EOC-induced CO_2-release equals EOC-addition?

We selected three discrete-compartment-models (described in the material and methods section)

D1	first-order	one compartment	$C(t) = C_s(1 - e^{-kt})$
D2	first-order	two compartments	$C(t) = \alpha\, C_s\left(1 - e^{-k_1 t}\right) + (1-\alpha)\, C_s(1 - e^{-k_2 t})$
D3	second order	one compartment	$C(t) = C_s - \frac{C_s}{1 + k\, a(1-a)\, C_s\, t}$

To test the robustness of the model towards different soil properties and EOC properties, the EOC-induced CO_2 release was measured in four soils differing in texture, and using two types of EOC with large differences in decomposition (straw, residues from anaerobic digestion of maize for biogas production). The suitability of six different models for simulating our data on CO_2-release was tested.

We hypothesized, that independently of soil and EOC, the two compartment model of first-order kinetics simulates the decomposition process best, as it is capable to illustrate altering substrate accessibility by two potentially biodegradable carbon compartments and reflects the limitation of EOC application in incubation experiments by considering the potentially biodegradable carbon amount to be lower than the initially applied carbon amount.

2.1.2 Decomposition of EOC as affected by different Soils and Soil types

Historically, humification has been defined as synthesis of humic substances (Scheffer et al., 1979), such as fulvic acids, humic acids, and humins (Olk and Gregorich, 2006), but recently overcome as stabilisation of soil organic matter by various environmental mechanisms towards mineralisation (Schmidt et al., 2011). The soil type influences two environmental mechanisms towards mineralisation of SOC: (i) Physical protection due to the adsorption onto clay mineral surfaces, amorphous iron, and amorphous aluminium (Kiem and Kogel-Knabner, 2002, Six et al., 2002). Soil organic carbon (SOC) decomposition enables the formation of stable aggregates (Watts et al., 2001, 2005). (ii) Physical disconnection of soil organic matter physically from decomposers, enzymes, and electron acceptors due to occlusion of organic matter in soil aggregates forms pores and structures, being inaccessible to microorganisms (Six et al., 2000).

Contrary to soil organic matter, being characterised by constant C: N: P: S ratio (Kirkby et al., 2011), EOC varies largely in the gravimetric proportion of C: N and further biochemical properties (Lashermes et al., 2009). The application of EOC, i.e. plant residues, farm fertiliser, digestates, and char, provides energy-rich carbon compounds to the soil microorganisms, inducing an initial increase in microbial activity, followed by gradually decreasing decomposition rates until a final stage with marginal energy-uptake into microorganisms (Ekschmitt et al., 2005). The decomposition process occurs over years in cultivation (Wessen and Berg, 1986), but can be observed within months in laboratory incubation (Sleutel et al., 2005). In agro-ecosystems, soil and site characteristics influence decomposition on numerous ways, as they control microbial growth (Treseder, 2008) and activity (Liu and Greaver, 2010) via plant growth (NPP), water-, nutrient-, and O_2-availability. It is therefore assumed, that soil type controls accessibility of soil organic matter to decomposing microorganisms (Dungait et al., 2012).

Incubation experiments standardise environmental conditions and the amount of plant residues, farm fertilisers, and amendments incorporated into soil (incubation ratio) to identify the effect of EOC application on microbial activity. One major question for the setup of incubation experiments remains the choice of an appropriate soil. In this chapter, we addressed to the following question: a) Does the EOC-induced CO_2-release and C_{pot} depend on the soil type? b) Is there an interaction between different EOC types and soil types in the determination of C_{pot}?

We measured the EOC-induced CO_2 release in four soils differing in texture, and using two types of EOC with large differences in decomposition (straw, residues from anaerobic digestion of maize for biogas production).

2.1.3 Influence of incubation temperature on decomposition

Persistence of SOC is controlled by temperature (Chen et al., 2013). The controlling mechanisms are complex and difficult to predict, as physical protection and decomposition of SOC differently respond to temperature changes (Conant et al., 2011): While increasing temperature favours adsorption of SOC to minerals at low-affinity, high-affinity associations are being desorbed. At higher temperature, microorganisms utilise accessible SOC less efficiently (CUE) and distribute more enzymes to soil. The enzymatic depolymerisation of organic matter therefore increases. As a result, persistence of SOC increases with geographical latitude, and decreasing temperature (Chen et al., 2013).

Temperature sensitivity of soil respiration, expressed as Q_{10}-value, is being affected by the soil microbial community, more frequently as enhancing than compensating response and effects of cooling remain reversible (Karhu et al., 2014). Furthermore, temperature sensitivity of soil organic matter decomposition essentially depends on substrate quality, i.e. molecular size or aromaticity, effectively requiring specific activation energy (Davidson and Janssens, 2006, Craine et al., 2010, Conant et al., 2011, Wagai et al., 2013). As EOC and SOC constitute contrasting substrate quality, the nature of microbial community level responses in EOC decomposition could not be derived. The temperature choice therefore faces another important issue of incubation experiments and a contentious discussion: As incubation experiments are conducted at different temperatures, some authors propose an incubation temperature that naturally occurs over long periods in soil (Sleutel et al., 2005), while others choose high temperatures (26-28 °C) to accelerate the decomposition process (Lashermes et al., 2009). Many authors overcome this issue by temperature functions, i. e. Arrhenius equations (Franko and Oelschlägel, 1995) and S-shaped functions (DeNeve et al., 1996), adjusting the observed decomposition rate mathematically to a common temperature. However, lower incubation temperatures have been shown reduce (Pal et al., 1975) or to enhance (DeNeve et al., 1996) decomposition of EOC (Karhu et al., 2014). The question arises, if experiments at different incubation temperature are comparable by simple temperature reduction functions as they do not account for enhancing or compensating microbial community level responses? This investigation aims to characterise temperature sensitivity of plant residue decomposition and to evaluate two temperatures, 22 °C and 6 °C for incubation, representing decomposition in spring/summer and autumn/winter. According to experiences in temperature-sensitivity of SOC decomposition under restricted N-availability by Karhu et al. (2014), we hypothesized an enhancing response of the microbial community on temperature and reversibility of cooling effects on plant residue decomposition by rewarming.

2.1.4 Influence of the SOC priming effect and C-limitation on decomposition

Soil respiration rates largely depend on recently applied EOC to soil (Conant et al., 2011, Schmidt et al., 2011, Birge et al., 2015). Soil respiration rates increase as induced by EOC application. If EOC is applied discontinuously to soil at large time intervals, soil respiration rates continuously decrease until

the next application of EOC (Liu et al., 2006, Creamer et al., 2011). The mechanisms controlling the duration of this EOC-induced increase in soil respiration have been shown not to depend on the amount of microbial biomass carbon (Kemmitt et al., 2008, Birge et al., 2015), but on the amount of available SOC (SOC content of soil) (Don et al., 2013). Physicochemical stabilisation of EOC through the integration into soil aggregates (Six et al., 2002) might be the main reason for decreasing soil respiration rates. The mechanisms controlling the extent of this increase depend on the amount and biochemical composition of EOC as well as on soil microorganisms, which respond on EOC application with an altered mineralisation of SOC (priming effect) (Kuzyakov and Domanski, 2000).

The capacity of soil organisms to decompose SOC is proposed to depend on the availability of low-molecular carbon compounds, which are easily accessible for microorganisms and decrease apparent energy-limitation of microbial activity (Ekschmitt et al., 2005, Fontaine et al., 2007). Carbon supply could therefore even decrease SOC contents (Fontaine et al., 2004). The persistence of soil organic matter is largely influenced by positive priming effects due to EOC supply to soil (Schmidt et al., 2011).

Several incubation experiments, focusing the determination of the fraction of EOC, which is incorporated into SOC (so called 'C_{pot}'), measure the difference between CO_2-release of a certain soil with and without homogeneously incorporated EOC (so called 'EOC-induced CO_2-release'). Thereby, the cumulatively measured EOC-induced CO_2-release describes a heterogeneous carbon pool, which could either originate from SOC or EOC. Recent investigations on plant residues and further types of EOC in incubation experiments assumed, that the priming effect could be neglected for the description of EOC decomposition and declared the EOC-induced CO_2-release as 'EOC mineralisation' (Jensen et al., 2005, Lashermes et al., 2009). Contemporaneously Lashermes et al. (2009) emphasize the importance of the initial CO_2-release in the first 3 days after incorporation of EOC (C_{3d}) as predictive parameter for the estimation C_{pot}.

In this study we addressed two questions: (i) How large is the priming effect in different stages of decomposition? (ii) Is decomposition of EOC in incubations studies constrained by low availability of energy sources for microbial activity?

For investigation of the first question we amended soil with ^{13}C-labelled wheat biomass to be able to differentiate between non-labelled SOC and labelled biomass C as sources of CO_2-C which was released during incubation. For investigation of the second question, we added glucose to the soil in the incubation experiment in which the soil was amended with either wheat biomass C or residues from anaerobic digestion of maize. It is well documented that the chemical composition of organic compounds may control activity of microorganisms involved in decomposition. Therefore, we tested two substrates with different composition to investigate "energy limitation" of decomposition. Our hypothesis was that glucose application should increase decomposition of residues from anaerobic digestions of maize for biogas production ("digestate") more than decomposition of straw. Glucose was

added 35 days after start of incubation. We expected that 35 days after start of incubation, the easily available C sources from the organic C which was added on day 0 and from SOC were largely depleted.

2.2 Material and Methods

2.2.1 Incubation experiments for simulation of EOC decomposition with different models

The evaluation of different models was conducted with results from three different incubation experiments. The first incubation experiment observed straw and maize digestate decomposition in different soils as described in section 2.2.2 on page 27. The second incubation experiment focused on the decomposition of different types of plant residues and is described in section 0 on page 64. The third incubation experiment contained different types and groups of EOC and is described in section 4.2.2 on page 98.

2.2.2 Incubation experiment (influence of different soils / soil types on decomposition)

2.2.2.1 Soils and site characteristic

Four soils from contrasting geological sites in Germany were chosen, representing agronomic regions with significant potential for bioenergy production (Rätz et al., 2011) (Table 1). The sites were mainly on agricultural research stations with arable field cropping, except for Lobenstein on a farm with perennial field cropping in the crop rotation. The sampling occurred alongside a 20 m long inner band of each field by three replications, representing the upper 30 cm of the plough horizon (A_p-horizon). Solely the fine soil was collected after careful two-step sieving with 5 mm and 2 mm mesh width. In order to avoid severe disturbance of soil microorganisms, soils were stored at field-moisture and 5 °C. For the site characterisation, soil texture was analysed by sedimentation (Köhn, 1928), pH-value was measured (according to DIN 19684) with a pH-meter (WTW Multiline® IDS), mineral N concentration was measured (according to DIN 19746), and subsamples were further pulverised (Retsch® ball mill). The pulverised samples were analysed for total carbon (EN 15936) and total nitrogen concentrations (EN 16168), using elementary analysis (elementar® varioMAX®) after dry combustion (Dumas, 1831). Furthermore, each site was characterised by biologically active time (BAT): As field moisture and temperature mainly influence microbial activity, mean annual temperature, precipitation, and the soil mineral fine fraction were used as regression variables, to characterise the actual environmental conditions by a period of decomposition time at hypothesized optimal conditions (Franko and Oelschlägel, 1995), e.g. a BAT of 9 days for the Lobenstein site means that the actual decomposition conditions over a period of 365 days correspond to optimal decomposition conditions over a period of 9 days. All used soils were loams with increasing clay and silt proportions in the order: Berlin, Lobenstein, Gießen, and Jena. The Lobenstein soil was the only soil with periodical alteration of annual and perennial cropping (perennial field grass), and was characterised by high carbon and nitrogen concentrations. The Berlin soil was fertilised for decades with high amounts of farmyard manure, and was equally characterised by high carbon concentration. The Jena site was influenced by calcareous

geological substrate (soil pH 6.4). Before incubation, the soils were stored for 10 days at 22 °C, to adapt soil microbial community to incubation conditions.

Table 1 Geographic position, precipitation, land use, and soil properties of the plough horizon (0-0.3 m) from sites associated with bioenergy regions (Rätz et al., 2011) in Germany. BAT biologically active time expresses the equivalent period of optimal decomposition conditions to one year uncontrolled cultivation-specific environmental conditions.

Site	Geographic position (Harvard)		Mean annual climate (DWD) Precipitation	Temperature	Land use
Berlin	52.466N	13.301E	591 mm	9.5 °C	Annual cropping
Lobenstein	50.501N	11.585E	871 mm	7.2 °C	Annual / perennial cropping
Gießen	50.626N	8.698E	666 mm	10.6 °C	Annual cropping
Jena	51.011N	11.648E	612 mm	8.8 °C	Annual cropping

Site	Texture (USDA)	Clay [%]	Silt [%]	Sand [%]	BAT [d]	pH	C [%]	N [%]	C/N
Berlin	Sandy loam	7	21	72	47	5.9	1.6	0.11	14.2
Lobenstein	Silt loam	20	54	26	9	5.5	2.8	0.26	10.7
Gießen	Silt loam	21	66	13	25	5.4	1.2	0.11	11.2
Jena	Silty clay loam	31	63	6	21	6.4	1.2	0.10	11.7

2.2.2.2 Setup of the incubation study

Laboratory incubation experiments, lasting 301 days, were carried out with two contrasting EOC: winter wheat straw (C/N-ratio 52) and maize digestate (C/N-ratio 5). After milling (RETSCH® SM 2000) or disperging (IKA® ULTRA-TURRAX) to 1 mm particle size, the EOC was applied to soil columns by an incubation ratio of 400 mg EOC in 100 g soil. Five replicates of soil columns with and without EOC were set (n = 5). Contrary to previous investigations (Henriksen and Breland, 1999, Jensen et al., 2005, Lashermes et al., 2009), mineral N concentration was adjusted to the common level of 20 mg N per kg soil (except the Lobenstein soil, which already contained 42 mg N per kg soil) to avoid nitrogen limitation of microbial activity. The incubation was conducted in soil columns of 1.1 g cm^{-3} bulk density. Incubation temperature was 22 °C. At the start of incubation, soil water content was adjusted to 20.8 ml (Berlin), 28.3 ml (Lobenstein), 23.3 ml (Gießen), and 24.7 ml (Jena) H_2O per 100 g soil, expressing 50 % of water holding capacity (ISO 16072) for each soil.

2.2.2.3 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.15 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.3 M HCl and phenolphthalein as indicator. Measurement dates were 1, 3, 7, 14, 21, 35, 56, 77, 98, 120, 162, 217, and 301 days after start of incubation. The decomposition of EOC was calculated as difference between evolved CO_2 from soil columns with and without EOC, and expressed

as EOC-induced CO_2-release in g C (g EOC)$^{-1}$. This approach is named "apparent" decomposition, as it integrates SOC priming and EOC mineralisation.

2.2.3 Incubation experiment (influence of different incubation temperatures)

2.2.3.1 Soil and site

Topsoil (0-30 cm) of the IOSDV long-term field experiment Berlin-Dahlem, fertilised with straw- green manure and optimal mineral N fertilisation at 110 kg N ha^{-1}, was sampled (details on page 119).

2.2.3.2 Setup of the incubation study

Maize stubble was cut into pieces of 1 mm particle size by milling (RETSCH® SM 2000) and applied to soil columns by an incubation ratio of 400 mg EOC in 100 g soil (n = 3). The incubation was conducted in soil columns of 1.1 g cm^{-3} bulk density. As previous investigation in temperature effects on SOC decomposition revealed interaction of temperature and nitrogen availability (Karhu et al., 2014), no mineral N was supplied to enforce this mechanism. At the start of incubation, soil water content was adjusted to 50 % of water holding capacity (ISO 16072), by adding 14.6 ml H_2O per 100 g soil. At 22 °C (ISO 16072), a set of soil columns with and without wheat straw was incubated, serving as control. An appropriate set was incubated at 6 °C until day of incubation 161, when it was reset to 22 °C.

2.2.3.3 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.2 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.4 M HCl and phenolphthalein as indicator. Measurement dates were 1, 3, 7, 14, 21, 35, 56, 77, 98, 119, 161, 175, 217, 259, 302, 343, 400, and 493 days after start of incubation. The apparent decomposition of EOC in soil was expressed as EOC-induced CO_2-release in g C (g EOC)$^{-1}$. This approach is named "apparent" decomposition, as it integrates SOC priming and EOC mineralisation.

2.2.4 Incubation experiment (influence of the SOC priming effect and C-limitation of microbes)

2.2.4.1 Soil and site

Topsoil (0-30 cm) of the IOSDV long-term field experiment Berlin-Dahlem, fertilised with farmyard manure, was sampled (details on page 119), containing the ^{13}C isotope at natural abundance ($\delta^{13}C$ – 27).

2.2.4.2 Plant labelling with ^{13}C

A pulse labelling greenhouse experiment was conducted, to produce ^{13}C-enriched plant materials (Bromand et al., 2001). Winter wheat was cultivated in Mitscherlich vessels (n = 3), containing 5.5 kg sieved (2 mm particle size) topsoil of Berlin Dahlem, and being fertilised with 1 g mineral phosphorus, 1 g mineral N, and 0.6 g mineral sulphur. The stable carbon isotope was applied as $Ca^{13}CO_3$ in two

consecutive pulses, the first at 22 days after sowing by 0.015 g ^{13}C per vessel, the second 49 days after sowing by 0.05 g ^{13}C per vessel. Shoots were harvested 104 days after sowing (BBCH 39).

2.2.4.3 Setup of the incubation study

The EOC, winter wheat shoot (C/N-ratio 22) with an abundance label of $\delta^{13}C$ 72.5 and unlabelled maize digestate (C/N-ratio 5) were applied to soil columns by 261 mg EOC (100 g soil)$^{-1}$ in case of wheat shoot and by 400 mg EOC (100 g soil)$^{-1}$ in case of digestate. The incubation was conducted in soil columns of 1.1 g cm^{-3} bulk density. Incubation temperature was 22 °C. At the start of incubation, soil water content was adjusted to 14.6 ml H_2O per 100 g soil, expressing 50 % of water holding capacity (ISO 16072).

After 35 days of incubation, glucose at a rate of 80 mg glucose-C (100 g soil)$^{-1}$ was applied to one set of soil columns. The $\delta^{13}C$ of glucose was – 10. Glucose was applied as 1.1 M aqueous solution by dropwise addition of 1 ml. An equivalent amount of water was supplied to the other set of soil columns.

The total number of soil columns for this incubation study was 24: 3 levels of EOC amendment (no, wheat shoot, maize digestate), 2 levels of glucose application (with and without), 4 repetitions.

For each treatment apparent decomposition of glucose was calculated as difference of cumulative CO_2-evolution between soil columns with and without glucose application, expressed as EOC-induced CO_2-release in g C (g EOC)$^{-1}$.

2.2.4.4 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.2 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.4 M HCl and phenolphthalein as indicator. Absorbed CO_2 of soil columns with labelled wheat shoot was precipitated as $BaCO_3$, which could be separated by filtration and drying. Measurement dates were 1, 3, 7, 14, 21, 35, 42, 56, 77, 98, 119, and 140 days after incubation start. As glucose was applied 35 days after start of incubation, the last 6 dates correspond to 7, 21, 42, 63, 84 and 105 days after application of glucose to one set of the soil columns. The apparent decomposition of EOC in soil was expressed as EOC-induced CO_2-release in g C (g EOC)$^{-1}$.

2.2.4.5 ^{13}C-analysis

Isolated $BaCO_3$ as well as soil, wheat shoot, and glucose were analysed for $\delta^{13}C$ [‰] at the centre for agricultural landscape research stable isotope laboratory. A Thermo-Finnegan Flash HT elemental analyser flash combusted the samples, converting carbon to CO_2. The sample gas was analysed by a Thermo-Scientific Delta V advantage isotope ratio mass spectrometer as described in (Kayler et al., 2011). The isotopic values are expressed in delta notation (in ‰-units), relative to VPDB (Vienna Pee Dee Belemnite). Analysis of internal laboratory standards ensured that estimates of the organic isotopic values were accurate within 0.1‰.

Table 2 Isotopic values $\delta^{13}C$ [‰] of soil, wheat shoot and glucose in the incubation experiment.

Material	**$\delta^{13}C$ [‰]**
Soil	-27
Wheat Shoot	72.5
Glucose	-10

2.2.5 Statistical analysis and modelling

2.2.5.1 Mathematical models for decomposition

Six mathematical models were used to interpolate and extrapolate the apparent courses of EOC-induced CO_2-release: The **D1 model**, following first-order kinetics, describes the decomposition of a single carbon pool which is potentially biodegradable in decomposition. The model is written as:

$$C(t) = C_s(1 - e^{-kt})$$

where t is the time in days, C_s is the potentially biodegradable pool in g C (g EOC)$^{-1}$, and k the decomposition rate in days^{-1}. The D1 model allowed the determination of the mean transit time τ_s (Manzoni et al., 2012a), characterising the persistence of the potentially biodegradable carbon pool C_s:

$$\tau_s = \frac{1}{k}$$

The **$D1_{EOC}$ model**, solely considers the case that the initially added EOC equals the total EOC-induced CO2-release in incubation:

$$C(t) = 1 - e^{-kt}$$

The **D2 model**, following parallel first-order kinetics, describe the decomposition of two biodegradable carbon pools in incubation experiments:

$$C(t) = \alpha\, C_s\left(1 - e^{-k_1 t}\right) + (1 - \alpha)\, C_s(1 - e^{-k_2 t})$$

where k_1 and k_2 are two different decomposition rates in days^{-1}, α marks the portion of the potentially biodegradable pool C_s which is decomposed at decomposition rate k_1, and t is the time in days. The D2 model allowed the determination of the mean transit time τ_s (Manzoni et al., 2012a), characterising the persistence of the potentially biodegradable carbon pool C_s:

$$\tau_s = \frac{(1 - \alpha)\, k_1 + \alpha\, k_2}{k_1\, k_2}$$

The **$D2_{EOC}$ model**, solely considers the case that the initially added EOC equals the total EOC-induced CO2-release in incubation:

$$C(t) = \alpha\left(1 - e^{-k_1 t}\right) + (1 - \alpha)(1 - e^{-k_2 t})$$

The **D3 model**, further following second-order kinetics, describes the decomposition of a single carbon pool which is potentially biodegradable in incubation experiments, but takes into account the conversion of EOC into microbial biomass carbon (Sleutel et al., 2005):

$$C(t) = C_s - \frac{C_s}{1 + k\,a(1-a)\,C_s\,t}$$

where *t* is the time in days, *k a (1-a)* is solely estimable as lumped value of both, the decomposition rate constant *k* and *a* the fraction of C_s, which is converted into microbial biomass.

The **$D3_{EOC}$ model**, solely considers the case that the initially added EOC equals the total EOC-induced CO2-release in incubation:

$$C(t) = 1 - \frac{1}{1 + k\,a(1-a)\,t}$$

2.2.5.2 Quality of model fitting

The six mathematical models were fitted to the complete observed courses of EOC-induced CO_2-release in Wolfram *Mathematica*® 10.2, using the Marquardt-Levenberg algorithm. According to previous investigation, the following statistical criteria were applied to evaluate the goodness of fit (Lashermes et al., 2009):

1 The coefficient of determination R^2, measuring the variation which could be explained by the model.

2 The root mean square error *RMSE*, showing the average difference between all observations and the modelled mean (Lashermes et al., 2009).

3 The root mean square error calculated for the last five measurement dates $RMSE_{end}$, focusing the deviation of the model from the final kinetics. This criterion evaluated the accuracy of C_{pot} estimations.

4 The difference of C_{pot} after 120 and 301 days of incubation C_{pot} *(301 d)*- C_{pot} *(120 d)*. The dependency on stage of incubation experiments was previously assumed as criterion for the precision of C_{pot} estimations (Sleutel et al., 2005, Lashermes et al., 2009).

2.2.5.3 Calculation of C_{pot}

The aim of the agricultural approach is the determination of humification efficiency as the portion of EOC which is transferred into the stable organic matter pool. For this reason, incubation experiments enable the calculation of C_{pot} in EOC, which is assumed to decompose like soil organic carbon (Lashermes et al., 2009). After the biodegradable carbon pool C_s was estimated by the proposed mathematical models for decomposition, C_{pot} was calculated as complement to C_s in the magnitude of EOC, indicated as g C (kg EOC)$^{-1}$:

$$C_{pot} = EOC - C_s$$

2.2.5.4 Mathematical model for long-term decomposition

Ecological carbon balancing concepts deal with carbon persistence in measurable ecosystem components. In this context not humification efficiency, but persistence of EOC matters (Schmidt et al., 2011). In this study, on the assumption that C_{pot} is being decomposed equally to soil organic carbon at a rate k_l of 0,02 year^{-1} (Lashermes et al., 2009), persistence of EOC in soil was estimated. Therefore, long-term decomposition was assumed to follow the simplified model of serial first order kinetics, which regards the decomposition of C_s at a rate of $1/\tau_s$ as the first step followed by subsequent decomposition of the (1-C_s) at the rate k_l. Thereof mean transit time τ of EOC was derived as follows (Manzoni et al., 2012a):

$$C(t) = 1 - \frac{(1 - C_s)\frac{1}{\tau_s}e^{-k_l t} - (k_l - \frac{1}{\tau_s}C_s)e^{-k_s t}}{\frac{1}{\tau_s} - k_l}$$

$$\tau = \frac{(1 - C_s)\frac{1}{\tau_s} + k_l}{\frac{1}{\tau_s}k_l}$$

Where $C(t)$ is the EOC-induced CO_2-release in g C (g EOC)$^{-1}$, C_s is the biodegradable carbon pool in decomposition, τ_s is the mean transit time of C_s, both estimated by the D2 model, and k_l is the assumed long-term decomposition rate of 0.02 year^{-1}. For this purpose the mean transit time of the potentially biodegradable pool τ_s was transformed into appropriate periods of cultivation time, following the concept of biologically active time as equivalence criterion (Franko and Oelschlägel, 1995). The biologically active time of one year incubation was calculated by temperature and moisture reduction functions (Franko, 1997) to be 120 days.

2.2.5.5 Calculation of temperature sensitivity of decomposition rates

Temperature sensitivity of apparent decomposition rates [g C (g EOC)$^{-1}$ d^{-1}] was calculated as Q_{10}-value, according to previous investigation (Karhu et al., 2014) by:

$$Q_{10} = \left(\frac{R(T_2)}{R(T_1)}\right)^{\frac{10}{T_2 - T_1}}$$

where $R(T_2)$ and $R(T_1)$ are decomposition rates at temperatures 22 °C and 6 °C, T_2 is control temperature 22 °C, and T_1 is cooling temperature 6 °C.

2.2.5.6 Calculation of SOC-priming effect

Soil organic carbon mineralisation and EOC mineralisation were calculated using mass balance equations (Fontaine et al., 2007):

$$C_{SOC}\,A_{SOC} + C_{EOC}\,A_{EOC} = C_T\,A_T$$

$$C_{SOC} + C_{EOC} = C_T$$

where C_{SOC} is the mineralised soil organic carbon, C_{EOC} is the mineralised EOC, C_T is the total mineralised carbon as CO_2-evolution, A_{SOC} is the ^{13}C-abundance in soil organic carbon, A_{EOC} is the ^{13}C-abundance in EOC, and A_T is the ^{13}C-abundance in precipitated barium carbonate.

The SOC priming effect (C_{PE}) was calculated as:

$$C_{PE} = C_{SOC}(with\ EOC\ application) - C_{SOC}(control)$$

where C_{SOC} is the mineralised soil organic carbon with and without EOC application.

2.2.5.7 Assessment of energy-availability in EOC mineralisation

Energy-limitation was tested by glucose application and determined by the ^{13}C-label of wheat shoot towards the natural ^{13}C-abundance in soil and glucose. Exogenous organic carbon (EOC) mineralisation (C_{EOC}) was therefore calculated using extended mass balance equations:

$$C_{SOC}\,A_{SOC} + C_G\,A_G + C_{EOC}\,A_{EOC} = C_T\,A_T$$

$$C_{SOC} + C_G + C_{EOC} = C_T$$

where C_{SOC} is the mineralised soil organic carbon, C_{EOC} is the mineralised winter wheat carbon, C_G is the mineralised glucose carbon, C_T is the totally mineralised carbon as CO_2-evolution, A_{SOC} is the ^{13}C-abundance in soil organic carbon, A_{EOC} is the ^{13}C-abundance in winter wheat carbon, A_G is the ^{13}C-abundance in glucose carbon, and A_T is the ^{13}C-abundance in precipitated barium carbonate.

2.3 Results

2.3.1 Simulation of decomposition by different mathematical model types

2.3.1.1 Simulation of decomposition for straw and maize digestate in different soils

The course of EOC-induced CO_2-release of wheat straw and maize digestate appeared at initially high rates, which were successively decreasing (Figure 6). While rates of EOC-induced carbon loss decreased in case of wheat shoot, they abruptly levelled off in case of maize digestate. For both sources of EOC, the D1, D2, and D3 models simulated the course of EOC-induced CO_2-release at visibly high goodness of fit, especially in the beginning of the incubation experiment. After 217 days of incubation, the D1 model underestimated the actual EOC-induced CO_2-release, whereas the D3 model slightly overestimated the actual EOC-induced CO_2-release of wheat shoot. The D2-model excellently simulated the courses of cumulative carbon loss for both maize digestate and wheat shoot.

In case of an assumed completeness of decomposition in the incubation experiment, each model deviated from the actual cumulative carbon loss in the end of the incubation experiment. This difference was low for wheat shoot decomposition, but high for decomposition of maize digestate. For maize digestate, the $D3_{EOC}$ model largely deviated from the actual course of EOC-induced CO_2-release in the beginning and in the end of incubation, while the $D1_{EOC}$ and $D2_{EOC}$ model failed to simulate maize digestate decomposition. The D1 – D3 models, which all estimate a potentially biodegradable pool of EOC, better followed EOC-induced CO_2-release than the $D1_{EOC}$, $D2_{EOC,}$ and $D3_{EOC}$ model.

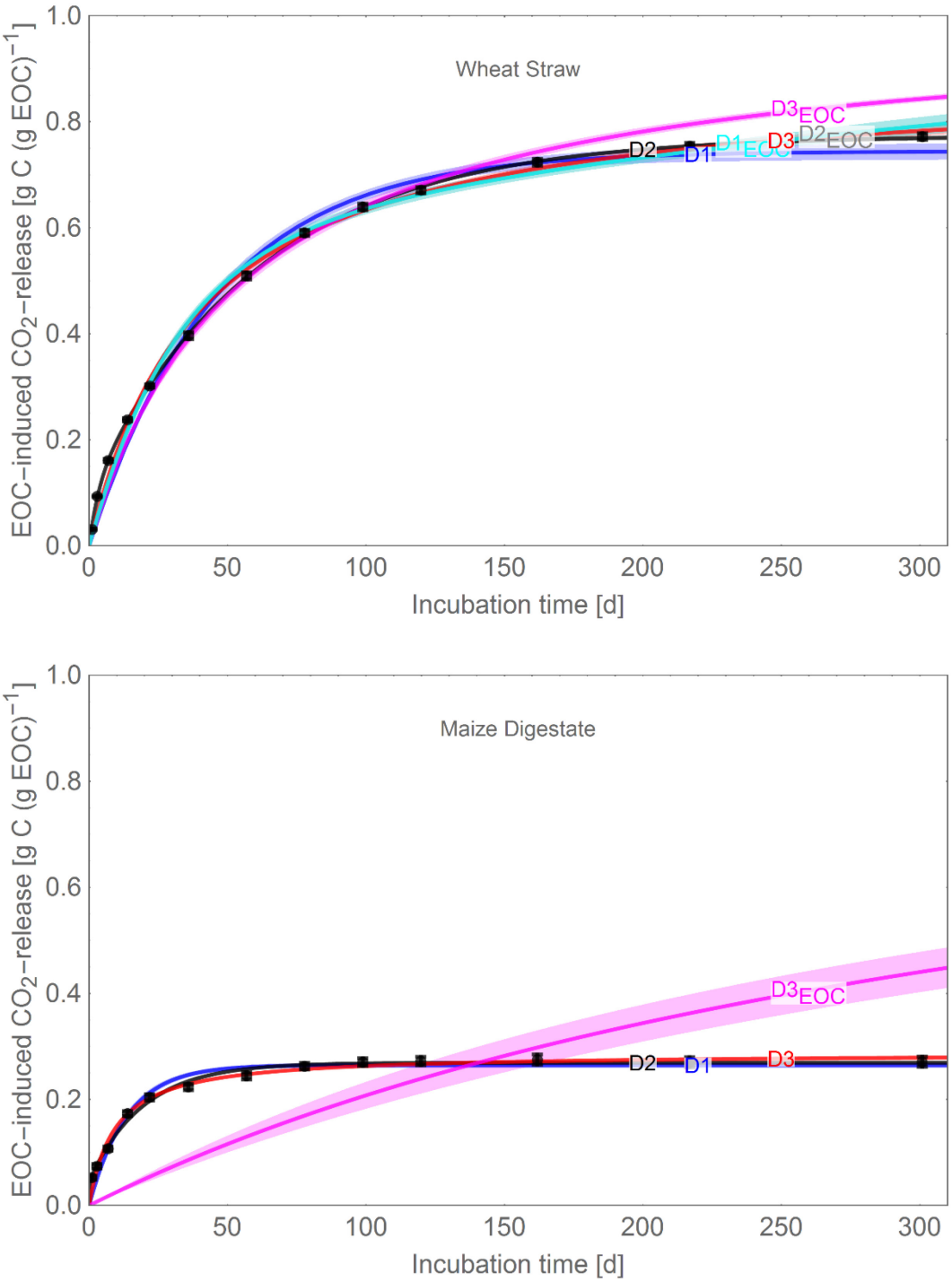

Figure 6 Simulation of decomposition for wheat straw in Berlin Dahlem soil by six different models: first order kinetic model with one carbon compartment D1 (blue) and two carbon compartments D2 (black), second order kinetic model D3 (red) and the $D3_{EOC}$ model (magenta, grey, and cyan). $D1_{EOC}$ and $D2_{EOC}$ models for maize digestate were not available. Black circles indicate measured means ± standard deviation (n = 5), model ± 95 %-CI (shaded). EOC Exogenous organic carbon

Across four different soil types the goodness of fit was highest for the D2 model, irrespective of EOC type (Table 3). The D2 model best fitted to the observed data, as root mean square error (RMSE) were lowest over the entire incubation duration and highest coefficients of determination could be reached, even under consideration of the number of variables in the model ($R_a^2 = 0{,}999$). The D3 model offered

similar goodness of fit in case of wheat straw, but not in case of maize digestate, whereas the D1 model came up with higher RMSE in both cases. The RMSE for the final four measurement occasions ($RMSE_{end}$) was lowest for the D2 model and therewith emphasized this model for accurate extrapolations and calculations of the potentially residual organic carbon pool C_{pot}.

The influence of incubation duration on the precision of C_{pot} estimations depended on the EOC type. In case of wheat straw, the precision of C_{pot} calculations from the D1 and D2 model largely depended on the incubation duration. The D1 model overestimated C_{pot} of wheat straw after 120 days of incubation by 52 g C (kg EOC)$^{-1}$, whereas the D2 model underestimated C_{pot} by 27 g C (kg EOC)$^{-1}$. The D3 model overestimated C_{pot} of wheat straw by 1 g C (kg EOC)$^{-1}$, remaining relatively robust towards different incubation durations. In case of maize digestate, the D2 model largely overestimated C_{pot}, whereas the D1 and D3 models remained relatively robust towards different incubation durations. Irrespective of the EOC source, the D2 model provided different C_{pot} estimations after different incubation durations, emphasizing the need for long lasting incubation experiments.

Table 3 Goodness of fit of the mathematical models D1, D2, D3, $D1_{EOC}$, $D2_{EOC}$, and $D3_{EOC}$ used to simulate decomposition and to calculate C_{pot} of wheat straw and maize digestate in four different soils (means ± standard deviation, n = 4, incubation n = 5): R_a^2 adjusted determination coefficient, RMSE root mean square errors between experimental and modelled values, $RMSE_{end}$ root mean square error between final experimental (120-301d) and modelled values, C_{pot} (301) – C_{pot} (120) difference between estimations of C_{pot} after two different periods of incubation, 120 and 301 days, respectively.

Model	R_a^2	RMSE	$RMSE_{end}$	C_{pot} (301) – C_{pot} (120)
		[g C (kg EOC)$^{-1}$]		
Wheat Straw (**plant derived EOC**)				
D1	0,995 ± 0,0016	32 ± 3	25 ± 4	-52 ± 16
$D1_{EOC}$	0,997 ± 0,0008	26 ± 2	22 ± 5	
D2	0,999 ± 0,0005	13 ± 5	16 ± 7	27 ± 47
$D2_{EOC}$	0,997 ± 0,0008	26 ± 2	22 ± 5	
D3	0,998 ± 0,0006	21 ± 2	18 ± 7	-1 ± 23
$D3_{EOC}$	0,984 ± 0,0085	57 ± 16	68 ± 20	
Maize digestate (**microbial processed EOC**)				
D1	0,978 ± 0,0168	30 ± 15	30 ± 22	-32 ± 38
$D1_{EOC}$	0,924 ± 0,1344	43 ± 48	40 ± 40	
D2	0,995 ± 0,0021	14 ± 4	15 ± 5	-736 ± 1452
$D2_{EOC}$	0,924 ± 0,1344	43 ± 48	40 ± 40	
D3	0,987 ± 0,0111	23 ± 12	25 ± 18	-32 ± 51
$D3_{EOC}$	0,821 ± 0,0681	88 ± 15	73 ± 22	

2.3.1.2 Simulation of decomposition for different plant residues

The course of EOC-induced CO_2-release of plant residues occurred at successively decreasing rates, which could be simulated in several mathematical models, converging towards a certain limit (Table 4). The courses of EOC-induced CO_2-release were best described by the D2 model ($R^2 > 0.989$ for each of the 40 plant residues, highest R^2 and lowest RMSE on average), which simulated first order kinetics of two biodegradable carbon pools at different decomposition rates. The D1 and D3 models, both regarding the evolved CO_2 as a single carbon pool, deviated more from observations during the entire period of incubation, but especially in the final sampling dates (higher RMSE and $RMSE_{end}$-values than D2 model). Therefore C_{pot} predictions of the D1 and D3 models were less accurate than C_{pot} predictions of the D2 model. However, the magnitude of the difference between C_{pot} predictions after 301 and 120 days of incubation was much higher for the D1 model than for the D2 and D3 models, indicating a lack of precision for the C_{pot} estimations from the D1 model.

Table 4 Goodness of fit of the applied models D1, D2, and D3 used to simulate decomposition and to calculate remaining organic carbon (C_{pot}) of plant residues in energy crop cultivation (means ± standard deviation, n = 40): *R^2* coefficient of determination, *RMSE* root mean square errors between experimental and modelled values, *$RMSE_{end}$* root mean square error between final experimental and modelled values (120-301 d), C_{pot} *(301)* – C_{pot} *(120)* difference between C_{pot} estimations after two different periods of incubation, 301 and 120 days, respectively.

Model	R^2	RMSE	$RMSE_{end}$	C_{pot} (301) – C_{pot} (120)
		[g C (kg EOC)$^{-1}$]		
D1	0.995 ± 0.004	35 ± 14	25 ± 9	-62 ± 45
D2	0.999 ± 0.002	14 ± 6	17 ± 8	17 ± 84
D3	0.996 ± 0.003	27 ± 1	20 ± 8	-11 ± 60

Referencing to the D2 model, which occurred to be the most accurate, the D1 model overestimated C_{pot}, whereas the D3 model underestimated $C_{pot,}$ especially for low C_{pot}-values (Figure 7). The D3 model evenly provided negative estimations. As negative C_{pot}-values could not be verified by longer periods of incubation (Figure 12), the D2 model was elected for the calculation of C_{pot}.

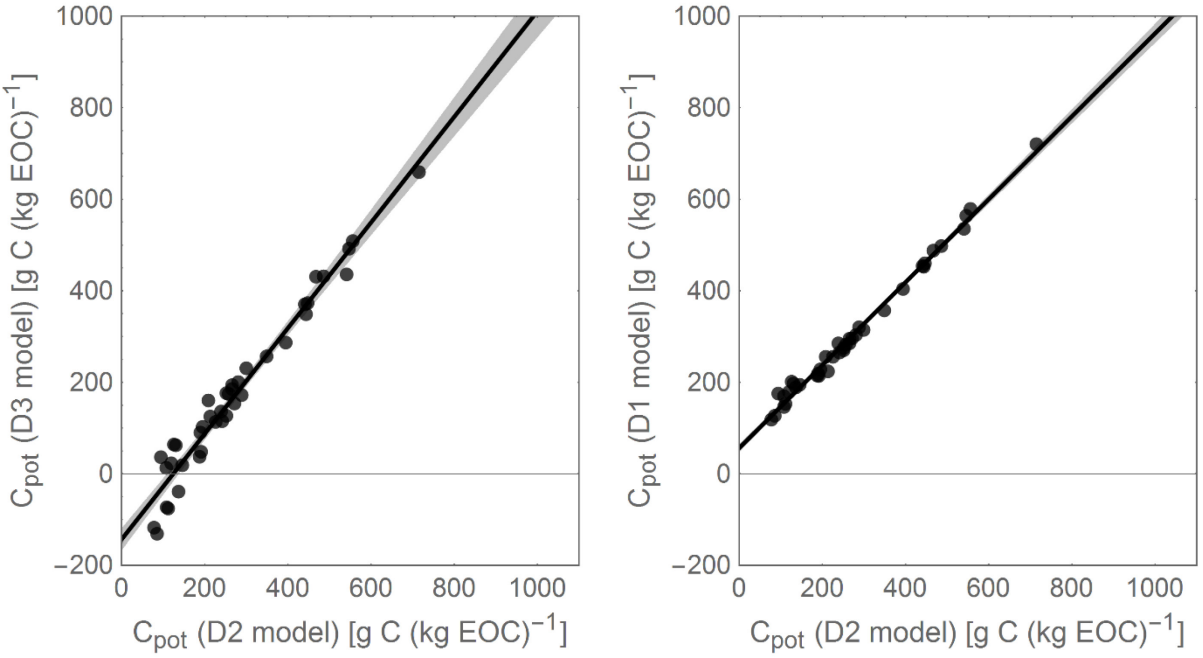

Figure 7 Potential residual organic carbon (C_{pot}) of plant residues calculated with D2 model versus calculations with D3 model (left) and D1 model (right). Linear regression ± 0.95 CI (n = 40).

Persistence of plant residues can be expressed as mean transit time, based on a fixed assumption of mean transit time for (0.02 year^{-1}) and the rate of decomposition (Figure 8). Mean transit time of plant residues τ was estimated to range in between decades, indicating large differences in the period of assumed long-term decomposition.

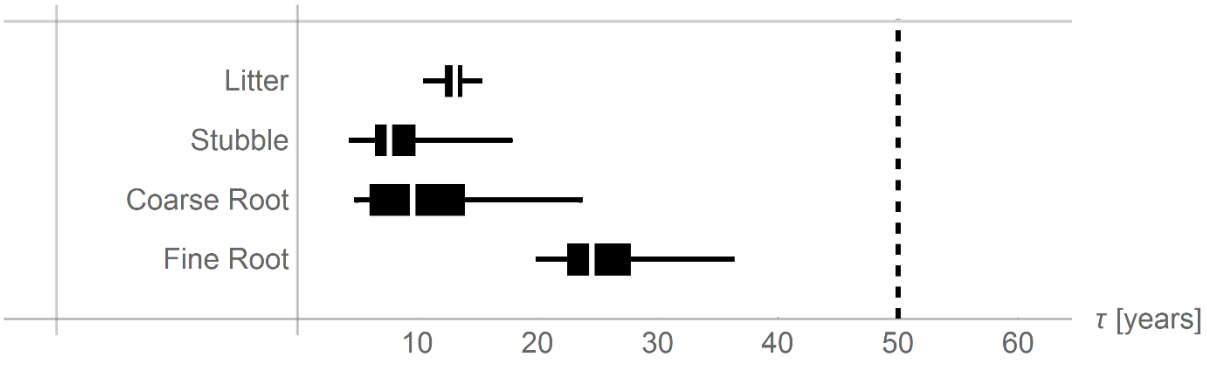

Figure 8 Distribution of mean transit time τ estimating persistence of 40 grouped plant residues in soil by serial first-order kinetics. The 10th and 90th percentiles (horizontal black lines), 25th and 75th percentiles (horizontal black boxes), median (white central vertical line). Black dashed vertical line indicates assumed mean transit time of soil organic matter.

2.3.1.3 Simulation of decomposition for differently processed EOC samples

The precision and the accuracy of estimations for C_{pot} depended on the applied model for simulation of the course of EOC-induced CO_2-release (Table 5). All three models, which were fitted to the course of EOC-induced CO_2-release, showed high coefficients of determination (R^2) across the magnitude of all 30 EOC samples. The RMSE for either all measured dates of cumulative EOC-induced CO_2-release or solely the final dates, which were measured from 120th to 310th day of incubation, were lowest for the D2 model and highest for the D1 model. Therefore first order kinetics (D1) did not more accurate simulate the course of EOC-induced CO_2-release than second order kinetics (D3), but the consideration of two biodegradable carbon pools in parallel first-order kinetic (D2) provided the most accurate simulation. Thus the D2 model provided the most accurate estimation of C_{pot}, which was even more accurate than the C_{pot} estimation of the D3 model. Whereas the accuracy of C_{pot} estimations depended on the goodness of fit of an applied model (R^2, RMSE, $RMSE_{end}$), the precision of the C_{pot} estimation depended on the concordance of C_{pot} estimations after different periods of incubation. However, C_{pot} estimations of the D3 model after 120 and 310 days of incubation differed by 11 ± 40 g C (kg EOC)$^{-1}$, and were lowest of all three applied models. The C_{pot} estimations of the D1 model after 120 days of incubation were on average 165 g C (kg EOC)$^{-1}$ higher than after 310 days of incubation. The D1 model therewith was the one of the least precise C_{pot} estimations. The D2 model was much more precise, as the difference of C_{pot} estimations between both periods of incubation was 38 g C (kg EOC)$^{-1}$, but the high standard deviation of 297 g C (kg EOC)$^{-1}$ revealed some extreme differences. Although the D2 model provided the most accurate C_{pot} estimations, it required the relatively long incubation duration of 310 days for precision.

Table 5 Goodness of fit of the applied models D1, D2, and D3 used to simulate decomposition and to calculate C_{pot} of EOC (means ± standard deviation, n = 30): *R^2* coefficient of determination, *RMSE* root mean square errors between experimental and modelled values, *$RMSE_{end}$* root mean square error between experimental and modelled values within 120 to 310 days of incubation, *C_{pot} (310)* C_{pot} estimation after 310 days of incubation, *C_{pot} (120)* C_{pot} estimation after 120 days of incubation.

Model	R^2		RMSE		$RMSE_{end}$		C_{pot} (310) – C_{pot} (120)	
			[g C (kg EOC)$^{-1}$]					
D1	0.965	± 0.1149	23	± 16	24	± 14	-165	± 101
D2	0.971	± 0.1090	13	± 6	15	± 8	38	± 297
D3	0.955	± 0.1181	17	± 9	17	± 9	-11	± 40

As the C_{pot} -estimations of the D1 or D3 model were less accurate than the ones of the D2 model, both specifically differed from the C_{pot} estmations of the D2 model (Figure 9). For low C_{pot}, the calculated values of the D3 model were lower than the values of the D2 model, whereas the ones of the D1 model were higher than the ones of the D2 model.

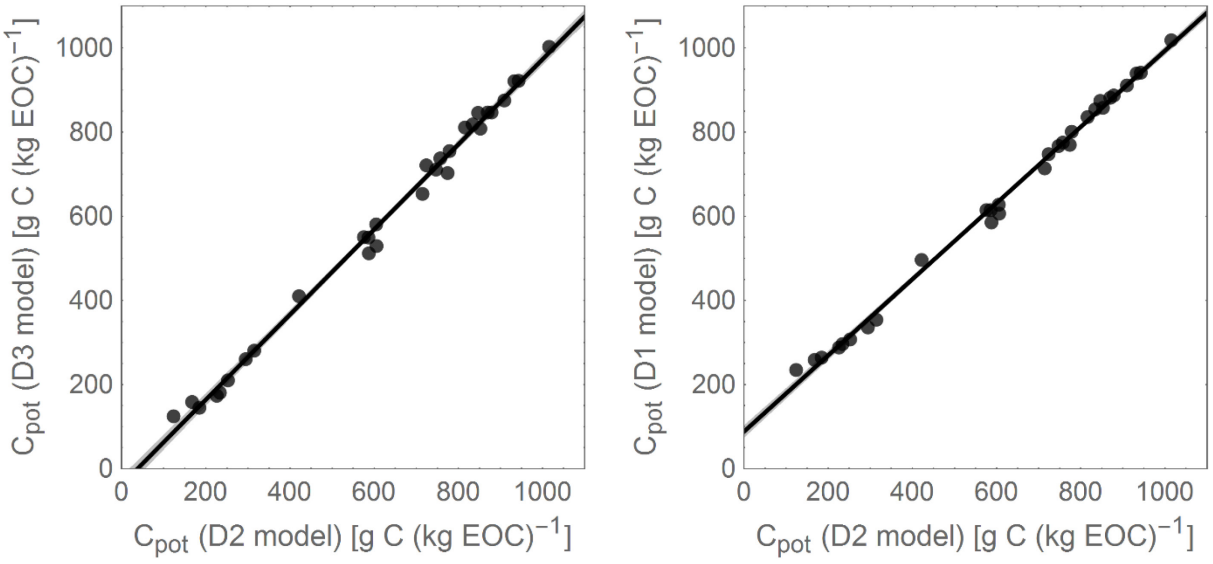

Figure 9 Relationship between C_{pot} of EOC calculated with D2 model versus calculations with D3 model (left) and D1 model (right). Linear regression ± 0.95 CI (n = 30).

C_{pot} and mean transit time τ_s of the potentially biodegradable carbon pool C_s were both used to estimate mean transit time τ of EOC in soil (Figure 10). However, both mean transit time τ of applied EOC and mean transit time τ_s of the potentially biodegradable carbon pool were not significantly correlated, and the persistence of potentially biodegradable carbon τ_s therewith remained obsolete for the estimated persistence of EOC. C_{pot} was tightly correlated to the estimated mean transit time τ of applied EOC, emphasizing the importance of C_{pot} for the estimation of persistence of EOC.

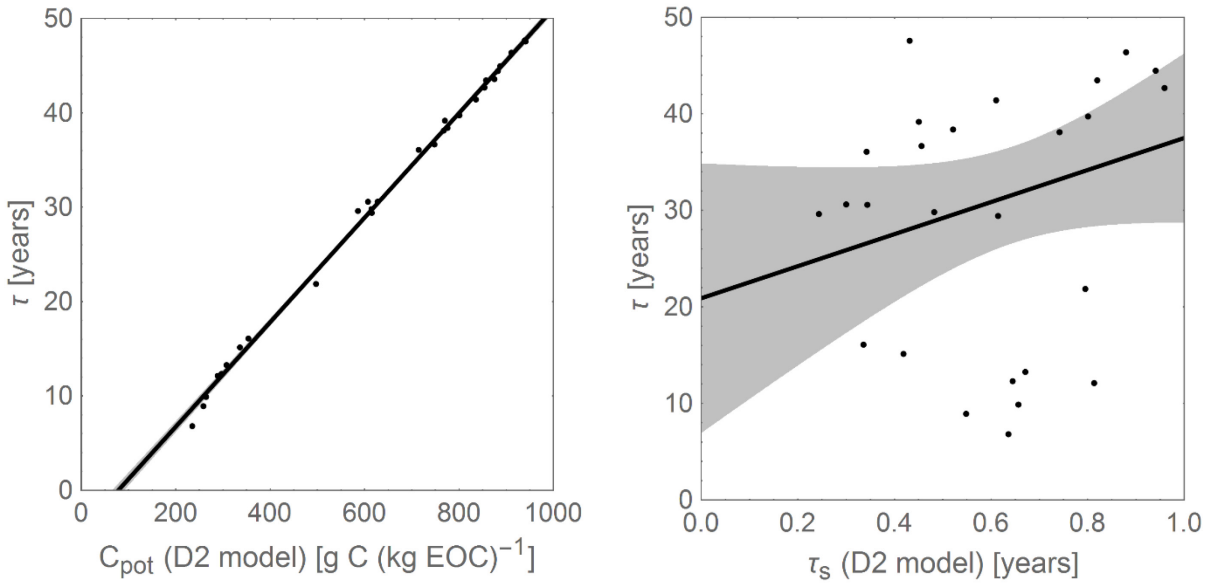

Figure 10 Relation between mean transit time τ of EOC, C_{pot} and the mean transit time of the potentially biodegradable carbon pool τ_s, respectively. Linear regression ± 95 % CI (shaded), n = 30.

2.3.2 Decomposition of EOC as affected by different Soils and Soil types

2.3.2.1 Influence of soil type on decomposition

The soil type significantly influenced the decomposition and C_{pot} of straw and digestate (Figure 11, Table 6). Differences between different soils occurred in case of wheat straw since 21st day of incubation, in case of maize digestate since 7th day of incubation, and manifested in later decomposition stages. An enhancement by coarse texture was obvious for wheat straw decomposition: The cumulative C loss was highest in the sandy loam of Berlin, intermediate in silt loams of Lobenstein and Gießen and lowest in the silty clay loam of Jena. A difference between decomposition in the silty clay loam of Jena and the the silt loam of Gießen solely existed intermediately, when microbial activity in the Jena soil was enhanced, presumably due to the higher pH-value in the Jena soil (lower Triassic). Furthermore, the higher N concentration in the silt loam of Lobenstein enhanced decomposition from the beginning on. Finally, decomposition in both silt loams ended in different C_{pot} of wheat straw, 278 g C (kg EOC)$^{-1}$ for Lobenstein and 334 g C (kg EOC)$^{-1}$ for Gießen, providing evidence for an influence of further soil specific physical protection mechanisms, i.e. adsorption onto Fe- and Al-oxides.

The cumulative C loss from maize digestate was much lower than from straw, proposing an enormous impact of initial biochemical quality on decomposition of EOC. As digestates are already microbial processed, they appeared to be more stable than straw. The soil type influenced the decomposition of digestate in a different way to the decomposition of wheat straw, indicating an interaction between soil and EOC type: Contrary to wheat straw, an increase of maize digestate decomposition by coarse texture was not obvious for all soils. Both silt loams showed increased decomposition since the 120th day of incubation. Finally, the course of EOC-induced CO_2-release significantly differed between both silt loams, as in the soil of Lobenstein C_{pot} 685 g C (kg EOC)$^{-1}$ was reached, while decomposition in the Gießen soil continued at constant rates.

Table 6 Potential residual organic carbon (C_{pot}; calculated with D2 model) of wheat straw and maize digestate in soils from four different sites in Germany.

Residue type	Site			
	Berlin	Lobenstein	Gießen	Jena
		C_{pot} [g C (kg EOC)$^{-1}$]		
Wheat straw	226 ± 5	278 ± 12	343 ± 10	334 ± 10
Maize digestate	731 ± 3	685 ± 15		789 ± 5

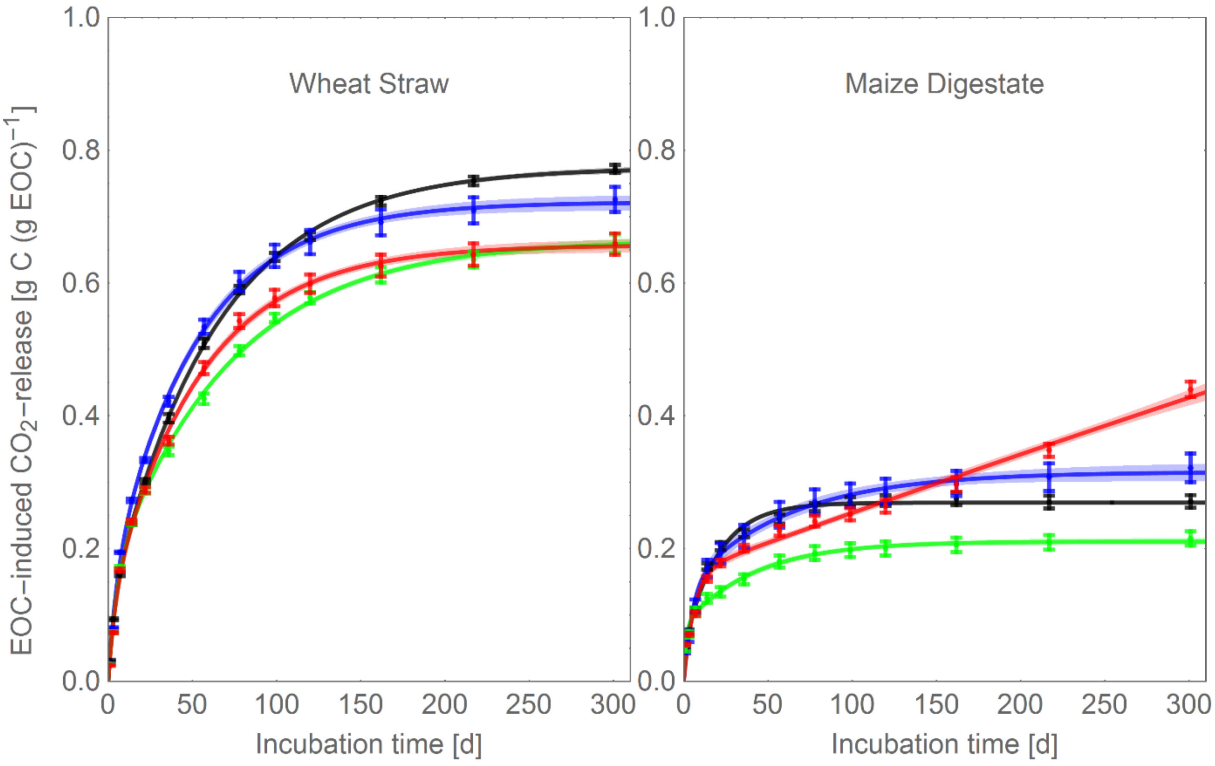

Figure 11 Decomposition of wheat straw and maize digestate in soils of four different sites: Berlin Dahlem sandy loam (black), Lobenstein silt loam (blue), Gießen silt loam (red), and Jena silty clay loam (green). Decomposition given as mean ± standard error (n = 5), and D2 model ± 95 %-CI (shaded).

2.3.3 Influence of incubation temperature on decomposition

2.3.3.1 Temperature sensitivity of plant residue decomposition

Incubation temperature significantly affected the course of EOC-induced CO_2-release of maize stubble in the period of cooling and subsequent rewarming (Figure 12). Decomposition of the control at 22 °C levelled out at 0.78 g C (g EOC)$^{-1}$, remaining incomplete and providing experimental evidence for a limitation of decomposition. Cooling decreased decomposition at successively lowering rates, seemingly being limited at 0.23 g C (g EOC)$^{-1}$. After cooling, crude particles still remained, obviously inaccessible to decomposition (Figure 14). Rewarming induced rehabilitation of microbial activity, and reconstitution of substrate accessibility, finally leading to a similar decomposition stage.

Temperature sensitivity of decomposition rates changed during the decomposition process (Figure 13). Within the second percentile of EOC-induced CO_2-release, the Q_{10}-value constantly increased from 1.8 to 3.2, indicating an increasing temperature sensitivity in parallel to decreasing carbon availability. The subsequent decomposition in the third percentile of EOC-induced CO_2-release was much more sensitive to temperature, as Q_{10} irregularly skipped to 6-7 and constantly remained at that level.

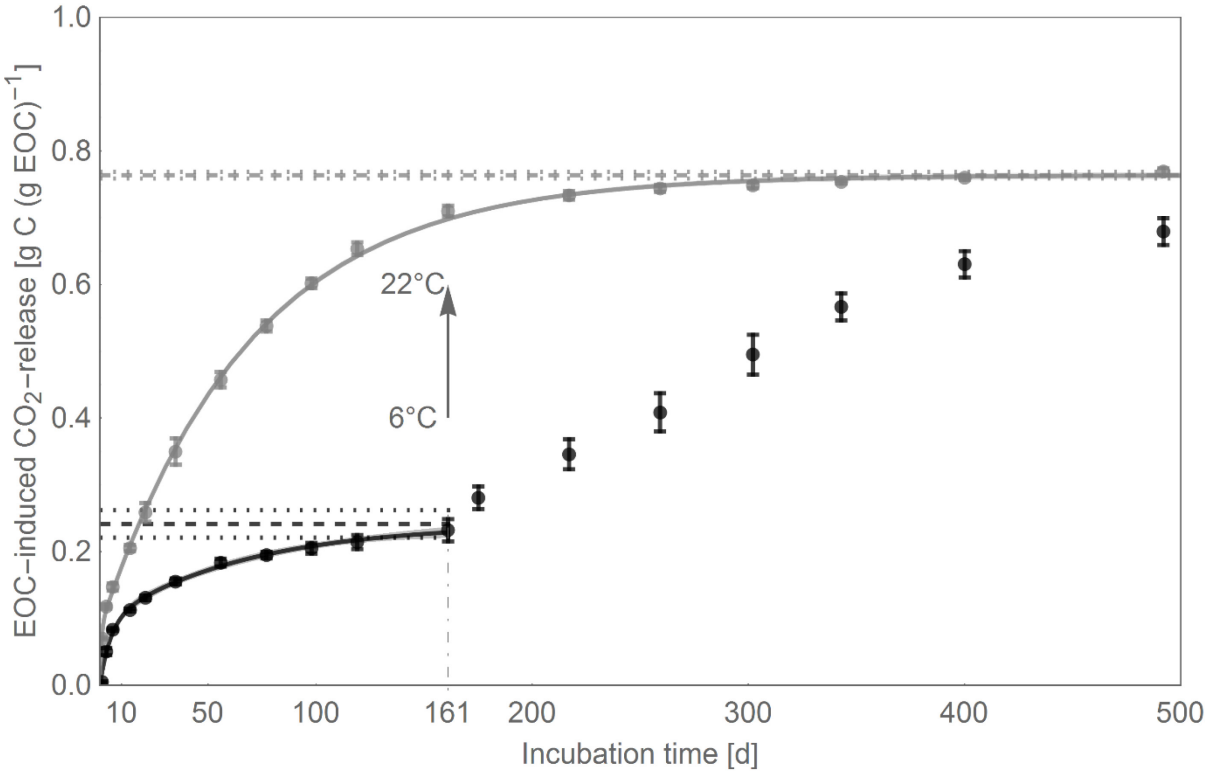

Figure 12 EOC-induced CO_2-release of maize stubble at 22 °C (grey) and at initially 6 °C followed by rewarming to 22 °C (black). EOC-induced CO_2-release given as mean ± standard error (n = 3), D2 model ± 95 % CI (shaded), and model limit (dashed) ± 95 % CI (dotted).

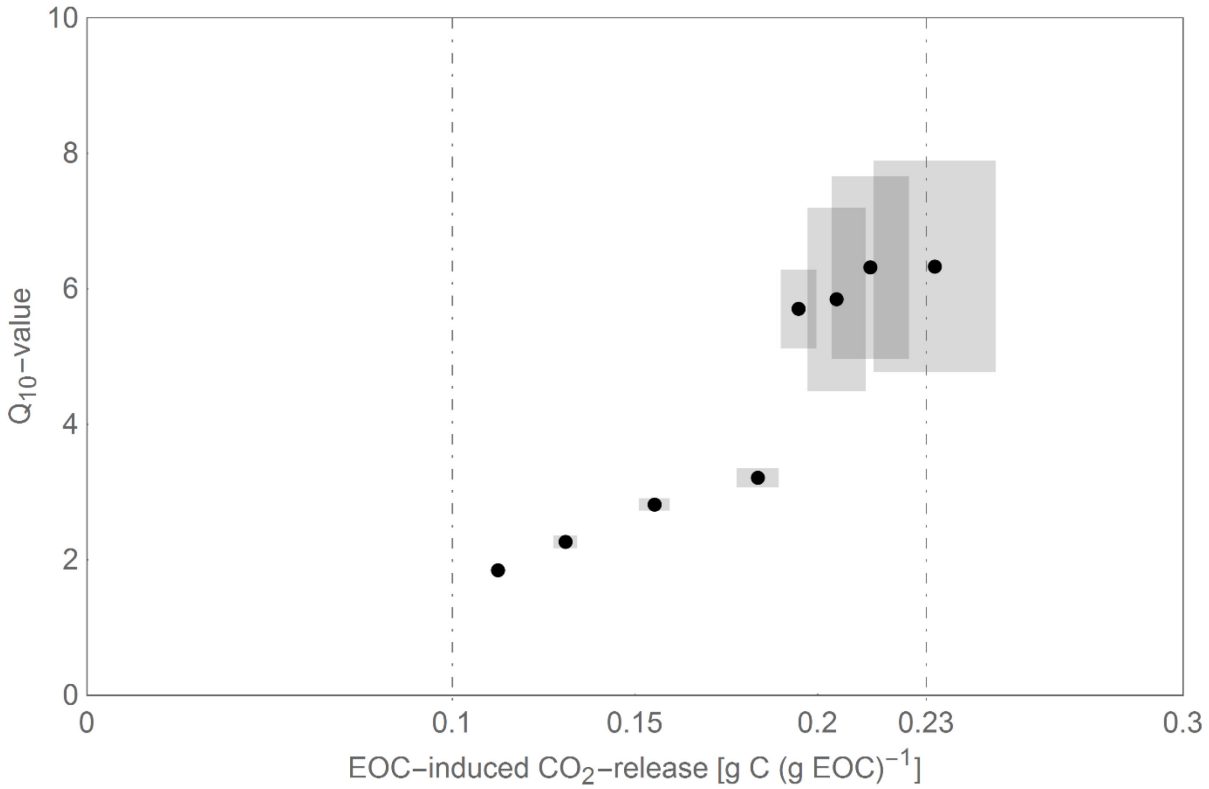

Figure 13 Q_{10}-value for decomposition rate of maize stubble at 6 °C and 22 °C by equal cumulative EOC-induced CO_2-release given as mean (black circles) ± standard error (shaded rectangles), n = 3.

The visual inspection of incubated soil columns after 161 days of incubation at different temperatures revealed less decomposed particles of maize stubble in the soil columns, which were incubated at 6 °C (Figure 14). Numerous plant particles in the outer layer of these soil columns visibly remained the initial particle structure and the original colour of the applied maize stubble. In contrast, the soil columns, which were incubated at 22°C appeared homogeneously brown and rarely showed plant particles in diminished particle structures, which had turned into brown during the decomposition process.

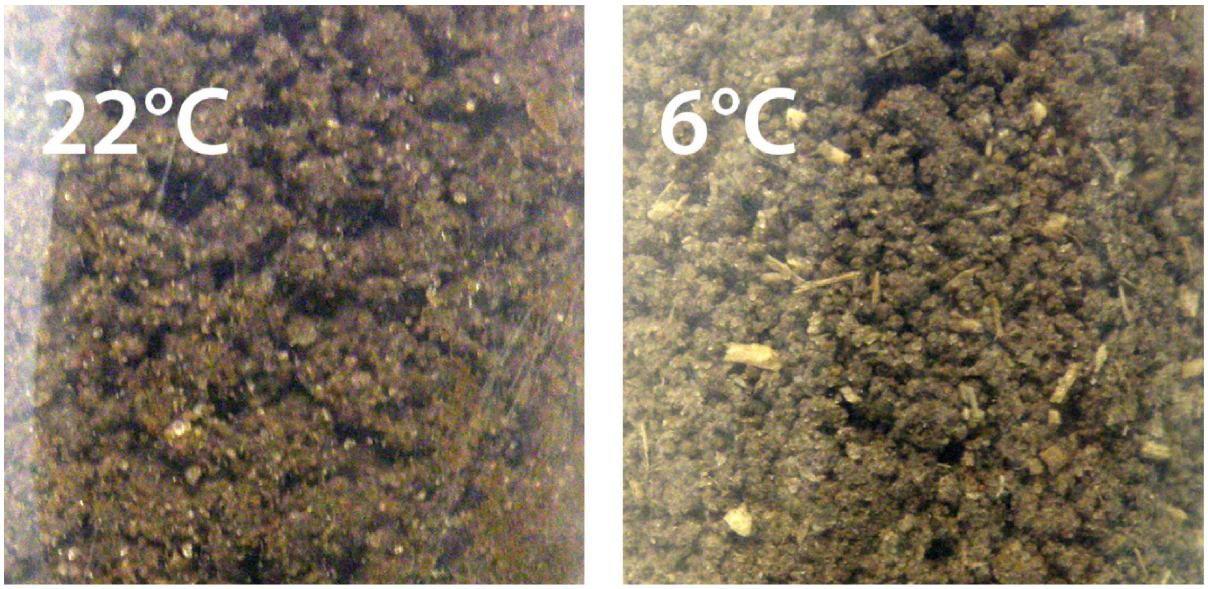

Figure 14 After 161 days of incubation at 6° C, coarse particles of maize stubble remained visible.

2.3.3.2 Microbial community-level response

The decomposition rate at equal stages of apparent decomposition revealed an enhancing microbial response at the community-level (Figure 15). Firstly, decomposition rates diverged, indicating enhanced temperature sensitivity of apparent decomposition by diverging microbial activity. Secondly, rewarming initially induced a little flush of CO_2-evolution within 14 days of incubation, as decomposition rate abruptly increased. Subsequently, decomposition rate slightly decreased, successively approximating control. Equal to the enhancing microbial community-level response on temperature sensitivity in soil organic matter decomposition (Karhu et al., 2014), consolidation occurred at lower decomposition rates.

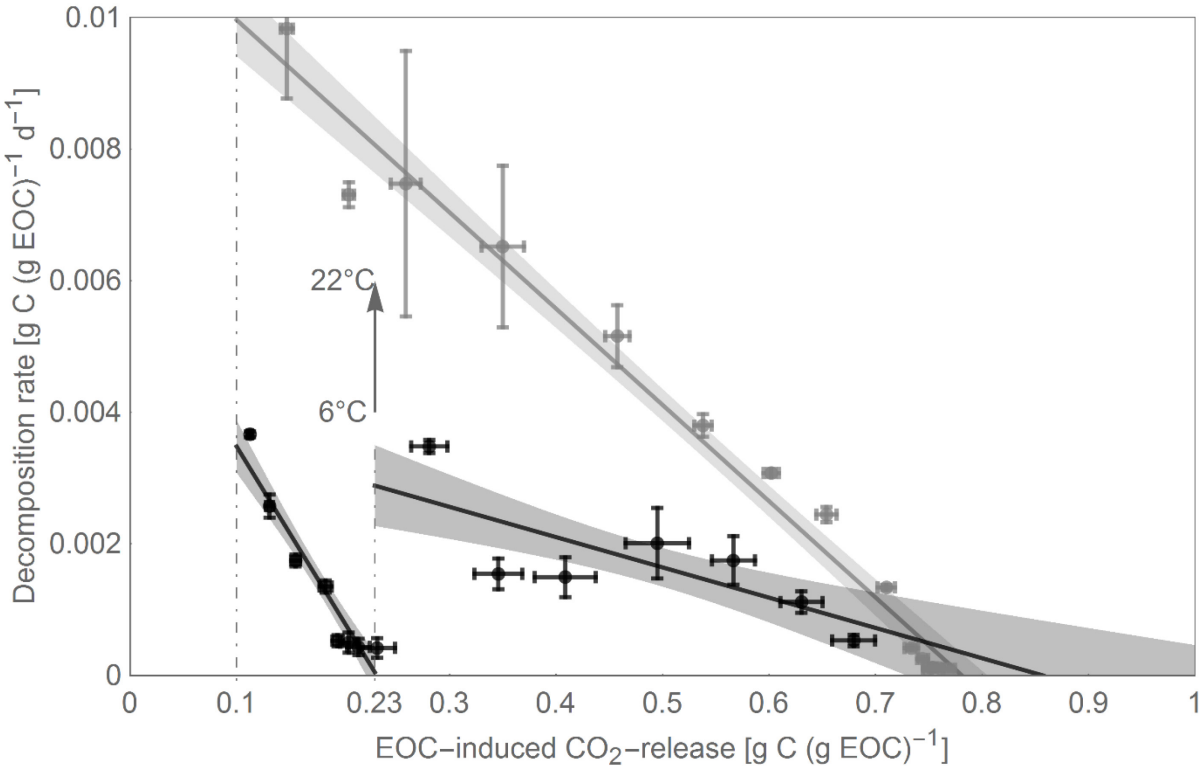

Figure 15 Enhancing microbial community-level response: Decomposition rate at 6 °C and after rewarming to 22 °C (black) compared to control at constantly 22 °C (grey). Decomposition rate of maize stubble given as mean ± standard error (n = 3), and linear model ± 95 % CI (shaded). EOC Exogenous organic carbon. The initial gap in the domain denotes that preincubation data are not considered.

2.3.4 Influence of the SOC priming effect and C-limitation on decomposition

2.3.4.1 Soil organic carbon priming by wheat shoot addition

Soil columns without amendment of fresh exogenous C released CO_2 during incubation at a low nearly constant rate (Figure 16, black symbols). As the soil did not contain $CaCO_3$, it may be assumed that CO_2 stemmed from SOC. During the 119 d of incubation, the steady CO_2-release accumulated to about 2 % (0.02 g g^{-1} SOC) of the initial SOC content. Soil columns amended with labelled fresh wheat shoot biomass released (unlabelled) CO_2 from SOC at a significantly higher rate (Figure 16, grey symbols). During the 119 d of incubation, the CO_2-release accumulated to about 10 % (0.1 g g^{-1} SOC) of the initial SOC contents. That is, CO_2 release from SOC was about 5 times higher in soil columns with than without shoot biomass amendment. The difference in SOC-derived CO_2 release between soil columns with and without shoot biomass amendment (Figure 16, blue symbols) shows the positive priming effect caused by shoot biomass amendment. A positive priming effect could be verified for the complete incubation duration (t-test, $P < 0.01$). The priming effect was particularly high in the first seven days and continuously decreased with increasing duration of incubation.

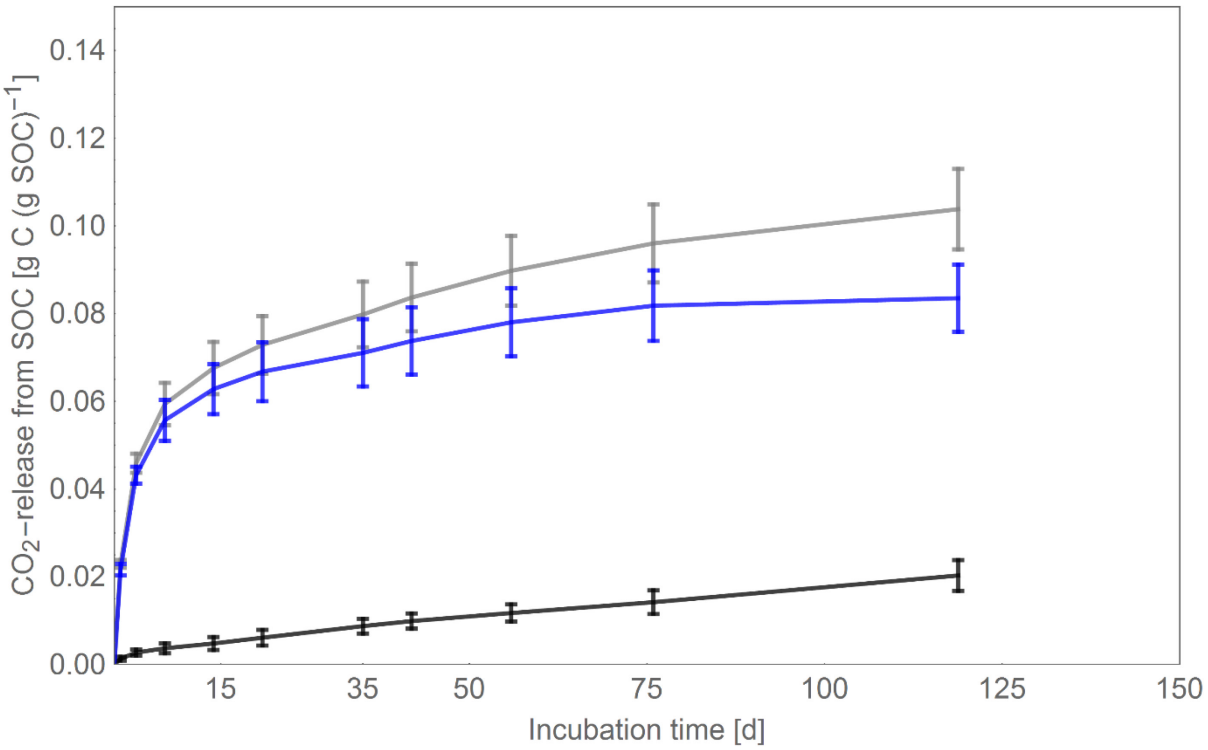

Figure 16 CO_2-release from SOC (SOC mineralisation) in the absence (black) and presence (grey) of wheat shoot application, and the resulting priming effect (blue) given as mean ± standard error (n = 4).

As wheat shoot application induced mineralisation of SOC (positive priming effect), the wheat-shoot induced CO_2-release has been differing from wheat-C mineralisation since the beginning of the incubation experiment (Figure 17). After the initial wheat shoot application, both wheat-induced CO_2-release and the mineralisation of wheat-C continued at decreasing rates, which seemed to converge towards a certain limit. After 100 days of incubation, the wheat-induced CO_2-release already accounted for 75 % of the carbon amount, which had been initially added as Wheat-C. The quantitative contribution of the priming effect was relatively high, so that actually less, about 55 % of the initially applied Wheat-C, had been released in the incubation experiment. The priming effect largely affected the Wheat-induced CO_2-release and therewith C_{pot} of wheat shoot.

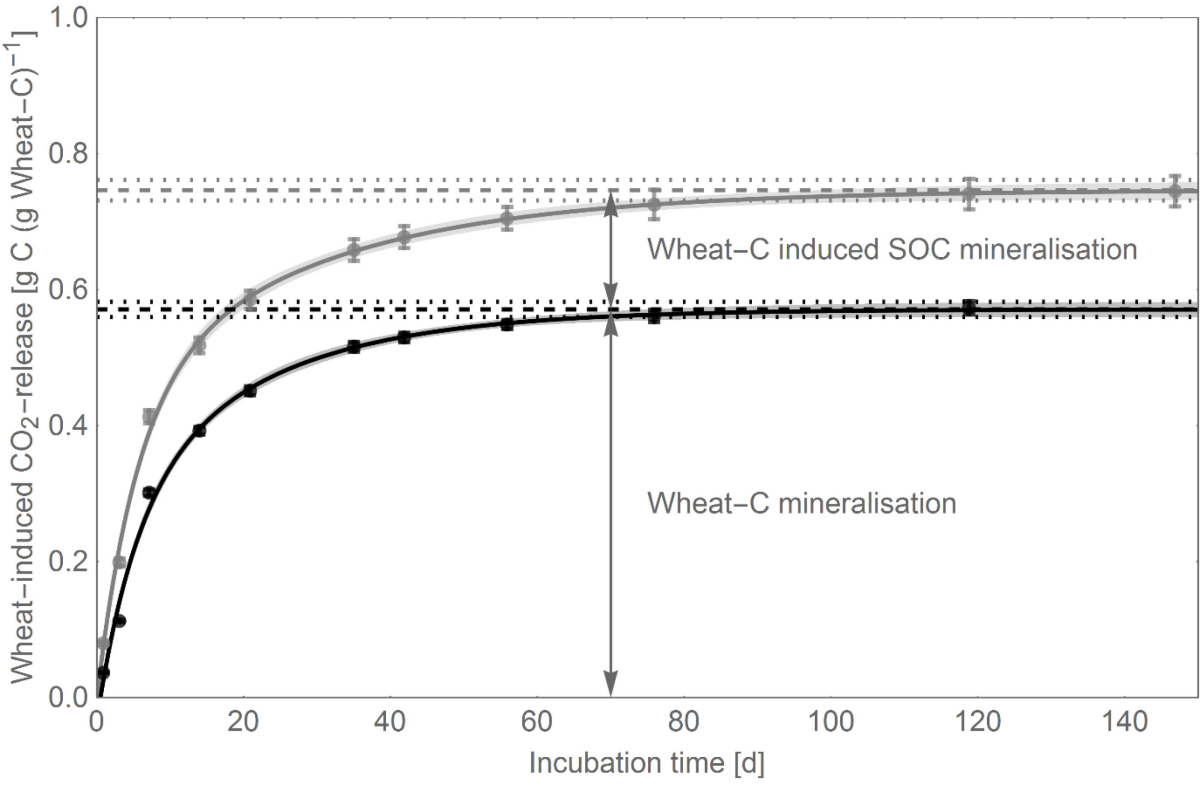

Figure 17 Wheat-induced CO_2-release (grey) and mineralisation of Wheat-C (black) given as mean ± standard error (n = 4), D2 model ± 95 % CI (shaded), and limit (dashed) ± 95 % CI (dotted).

2.3.4.2 Energy-limitation of exogenous organic carbon decomposition

The CO_2-release from soil columns was increased by the application of wheat shoot and maize digestate, but then occurred at successively lowering rates (Figure 18). 35 days after start of incubation, the addition of glucose modified the CO_2-release from soil columns for a second time. Within the first 7 days after glucose application, CO_2-release was drastically increased, 21 d after glucose application the cumulative CO_2-release was enhanced by about 50 mg C. The glucose-induced increase of CO_2-release was very similar in soil columns without and with amendments of shoot biomass or digestate. The differences in cumulative CO_2 release among the three soil treatments (without amendment, amended with shoot biomass, amended with digestate) were very similar irrespective of whether glucose was applied or not applied.

The glucose-induced CO_2 release was higher (t-test, $P < 0.01$) in soil amended with shoot biomass than in soil without amendment or in soil with digestate amendment, providing evidence for an apparent constraint of available carbon for the decomposing microorganisms in soil amended with wheat shoot, whereby this effect was essentially confined to the first 7 days after glucose application (Figure 19).

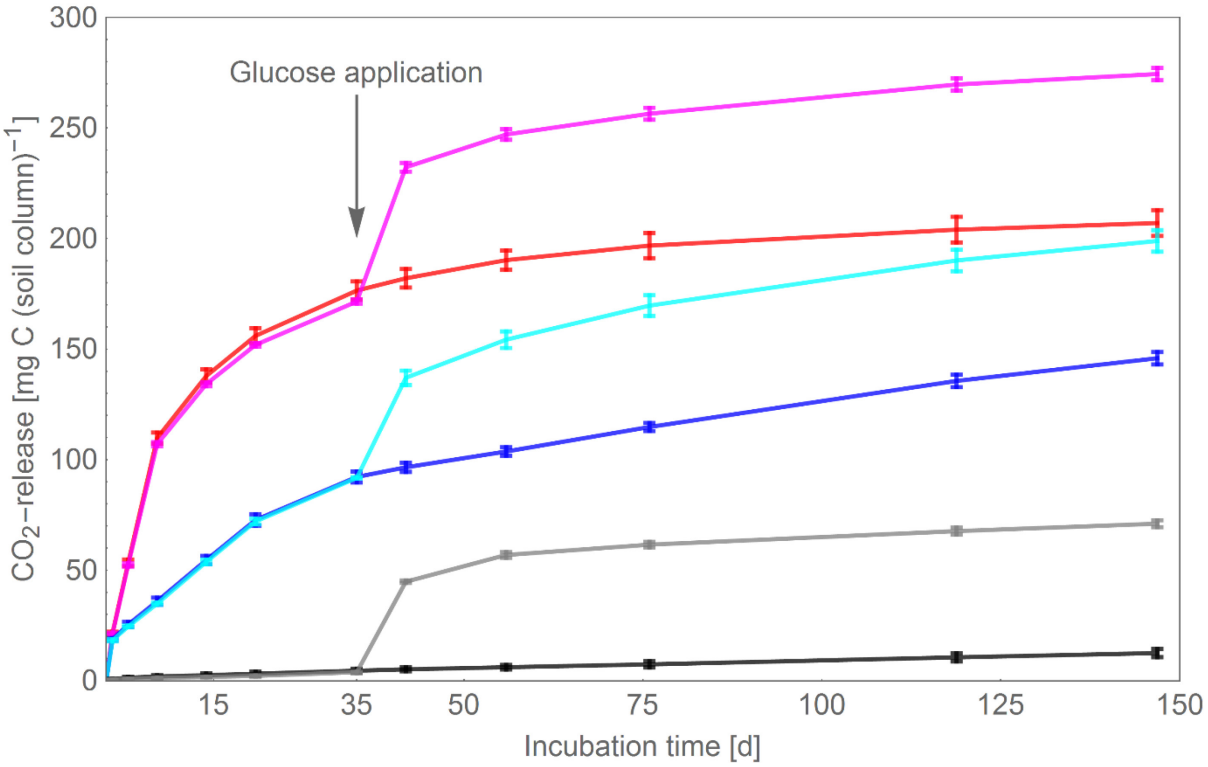

Figure 18 CO_2-release from soil columns with different carbon applications: without (black), with glucose (grey), with maize digestate (blue), with maize digestate and glucose (cyan), with wheat shoot (red), with wheat shoot and glucose (magenta) given as mean ± standard error (n = 4).

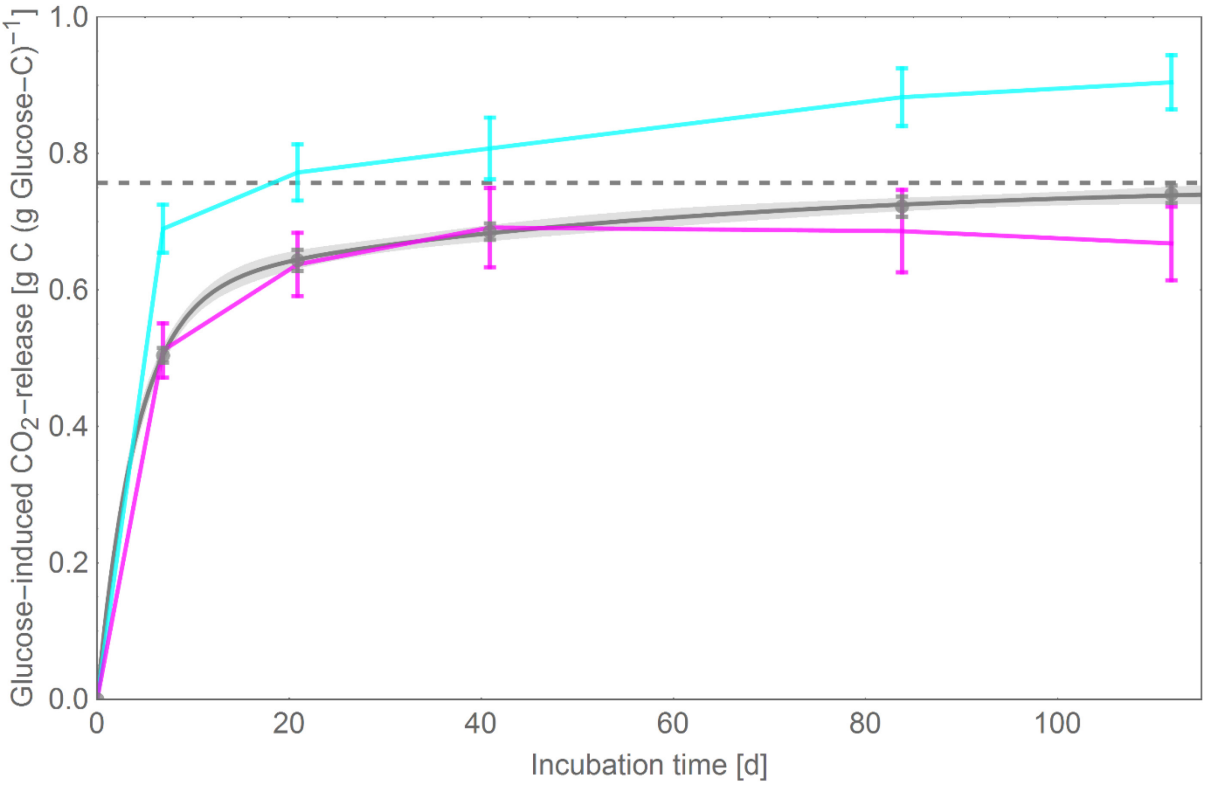

Figure 19 Glucose-induced CO_2-release after 35 days of preincubation with wheat shoot (magenta), maize digestate (cyan), or omitted carbon application (grey), given as mean ± standard error (n = 4), D2 model ± 95 % CI (shaded).

The CO_2-release from wheat shoot (wheat shoot mineralisation) was increased by the application of glucose until the 56th day of incubation at a rate of 0.012 g C (g Wheat-C)$^{-1}$ (Figure 20). Therefore, the decreased C_{pot} could be identified as an increased mineralisation of wheat shoot due to glucose addition.

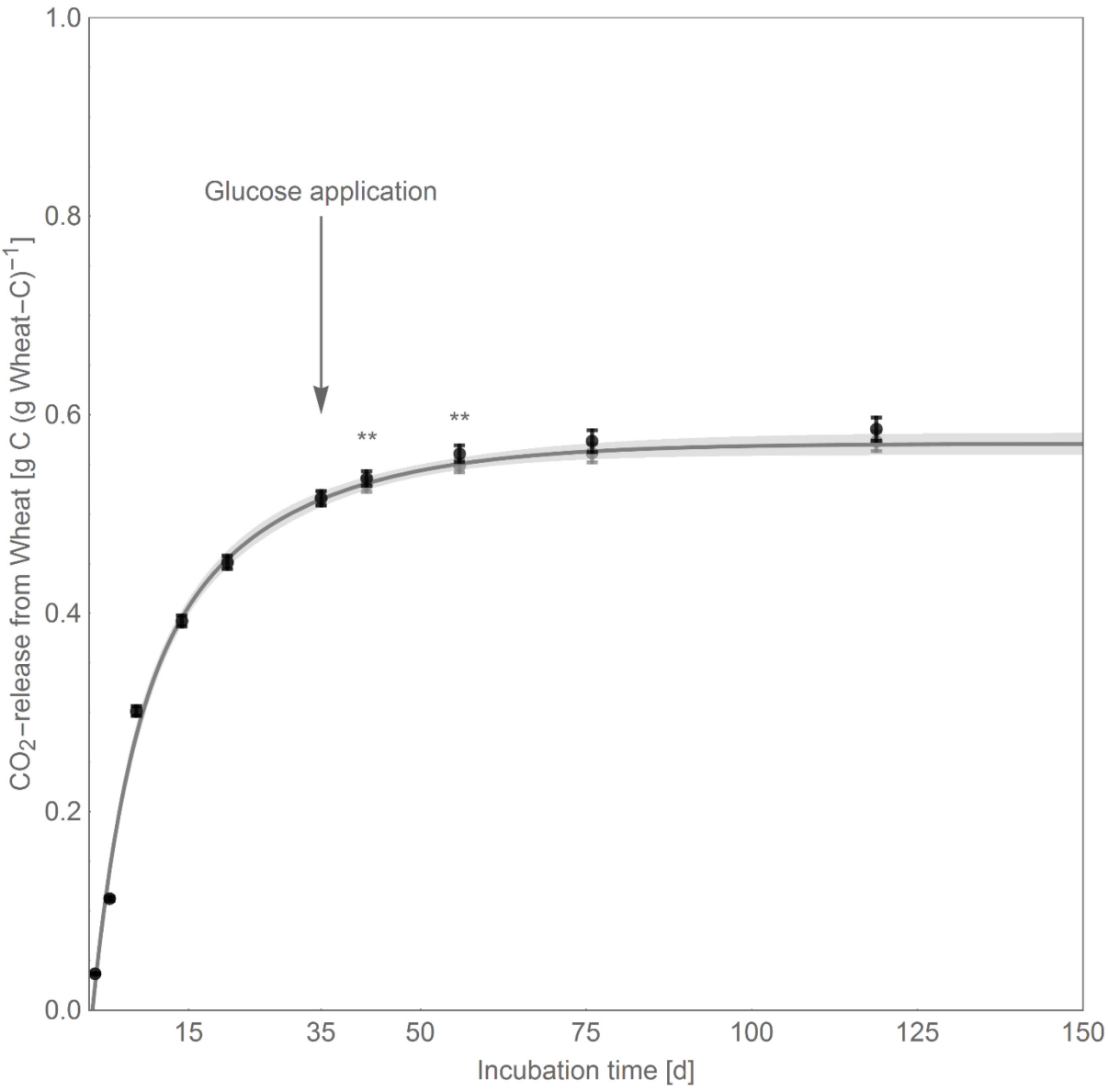

Figure 20 Mineralisation of wheat shoot in two identical incubation sets (grey / black symbols), whereby glucose was applied to one set (black symbols) after 35 days of incubation. CO_2-release from wheat shoot given as mean ± standard error (n = 4), D2 model ± 95 % CI (shaded). **indicate significant differences (Paired T-test, P < 0.01).

The influence of glucose application on the Wheat-induced or digestate-induced CO_2-release was relatively low and did not seriously alter C_{pot} of these EOC sources (Table 7). Glucose application slightly decreased C_{pot} of wheat shoot from 0.25 to 0.22 g C (g Wheat-C)$^{-1}$, whereas C_{pot} of digestate remained indifferent at 0.69 g C (g digestate-C)$^{-1}$.

Table 7 Influence of glucose application after 35 days of incubation on potential residual organic carbon (C_{pot}) of wheat shoot and maize digestate. C_{pot}-values given as mean ± 95%-CI, different characters indicate significant (at the 0.05 probability level) differences between C_{pot}-values.

Exogenous organic carbon (EOC) application	Glucose application after 35 days of incubation	
	– Glucose	+ Glucose
	C_{pot} g C (g EOC)$^{-1}$	
Wheat shoot	0.25 a ± 0.015	0.22 b ± 0.014
Maize digestate	0.67 c ± 0.011	0.69 c ± 0.009

2.4 Discussion

2.4.1 Simulation of decomposition by different mathematical model types

The results showed the best fit and the most accurate estimation of C_{pot} for the D2 model across different soils (Table 3), different plant residues (Table 4), and different EOC types (Table 5). In contrast to the D1 model, the D2 model allowed for the complexity of apparent decomposition in incubation experiments by the characterisation of two biodegradable carbon pools with different decomposition rates. In case of 120 days incubation duration, the D2 model underestimated C_{pot} of several EOC types (Table 4, Table 5), while the D3 model already provided reliable C_{pot} values. Therefore, the D3 model constituted an equal alternative, which should be preferred for incubation experiments of short duration.

Each model could be parameterised under the assumption, that EOC-induced CO_2-release equals the initial EOC input. The results showed no evidence for a better goodness of fit of these models (Table 3), neither to the course of cumulative carbon loss from wheat straw nor from maize digestate. The course of cumulative carbon loss, observed over 301 days of incubation, occurred at successively lower rates and finally levelled off depending on the source of EOC. This provided evidence for an incomplete decomposition irrespective of incubation duration. As incubation experiments face decomposition in a comparative approach of samples with and without EOC application, a stagnation in apparent decomposition does not indicate the end of microbial activity, but equal decomposition rates of soil with and without EOC. The early end up of apparent decomposition therefore provides evidence for stabilisation of EOC in soil as apparent issue of microbial activity and soil inherent properties, partially protecting and disconnecting EOC physically (Schmidt et al., 2011). In this way, the C_{pot} does not equal SOC in its biochemical quality and physicochemical structure, but in the accessibility to microorganisms (Dungait et al., 2012). The implication for actual accessibility under cultivation-specific conditions thereby remains uncertain, as both biology of microbial communities and biochemical quality determine accessibility beneath environmental stabilisation mechanisms (Ekschmitt et al., 2005).

2.4.2 Decomposition of EOC as affected by different Soils and Soil types

The collected soils represented different loams, specifying intermediate soils as typical for energy crop cultivation. The results showed, that different soil types, land use, and site conditions provided soils with various amounts of soil organic carbon to incubation experiments. The identification of cultivation

specific effects remains contentious, as mechanisms of soil type specific physical protection of soil organic matter (Kiem and Kogel-Knabner, 2002, Six et al., 2002), and further environmental conditions, i.e. different mean annual climate (Carvalhais et al., 2014) could explain variation in SOC concentrations likewise. In contrast of SOC concentrations, C:N-ratio remained at a virtually constant level (Kirkby et al., 2011), ensuring the comparability of SOC independently of soil type, site conditions and land use. Even extraordinary cultivation activity, like intensive farmyard-manure fertilisation in Berlin, and field grass cropping in Lobenstein, providing EOC sources in excess, did not affect C:N-ratio.

The selected soil importantly influenced apparent decomposition, and even the modelling of the courses of EOC-induced CO_2-release for the two contrasting EOC types wheat straw and maize digestate. According to the proposed mechanisms physically protecting soil organic matter (Schmidt et al., 2011), the involved soil properties associated to texture/soil type prominently regulated EOC decomposition: As the decomposition rates began to decrease after an excessive initial CO_2-evolution, the decrease was slight in coarse textured soils, providing support for altered accessibility of EOC (Dungait et al., 2012). Further soil properties influenced microbial activity, like the pH-value and nitrogen availability (Treseder, 2008), overlaying soil type specific effects on decomposition. While the courses of EOC-induced CO_2-release diverged after 35 days of incubation for straw, and even earlier for maize digestate according to the soil type, the soil of Lobenstein, containing the double amount of mineral N, showed enhanced straw decomposition since the beginning of the incubation experiment. Contrary to the immediate nature of nitrogen effects (Henriksen and Breland, 1999), the higher pH-value in the soil of Jena enhanced straw decomposition in the latter part and finally even neglected the effect of a fine texture. This provides support to the theory that accessibility of EOC also depends on biological issues of the microbial community: After microbial degradation, most organic carbon remains passively stabilised in dead organic matter and physical stabilisation remains labile (partial refuges), until altered nutrient availability enables K-strategists to dominate the microbial community and open it (Ekschmitt et al., 2005). Enhanced nitrogen availability and higher pH-values both improve the microbial habitat, and therefore enable the acquisition of potentially accessible carbon. Another – yet unidentified – mechanism enhanced the decomposition of maize digestate in both silt loams. If the magnitude of C_{pot} remains in such a passive stabilisation – partial refuge state, this result provides evidence for soil specific mechanisms to enhance the accessibility of digestates and microbial pre-processed EOC, opening up the partial refuge. In case of the silt loam of Gießen, the decomposition appears to continue at finally increasing decomposition rates, disqualifying this soil for the purpose to identify a course of EOC-induced CO_2-release in incubation experiments.

Although the soil type and other soil properties affected courses of EOC-induced CO_2-release in various periods on the observed time-scale, both contrasting EOC types wheat straw and maize digestate could be distinguished in the magnitude of collected soils. This identified incubation experiments as predestined methodology, to study the influence of biochemical properties on course of EOC-induced

CO_2-release of EOC, to identify responses of microbial activity and decomposition on nitrogen availability, and to describe implications for persistence of EOC in soil.

2.4.3 Influence of incubation temperature on decomposition

The results showed an enormously decreased decomposition of maize stubble by lowered incubation temperature. The magnitude of this decrease was bigger than reported before (Pal et al., 1975) and increased with ongoing decomposition in contrast to an reported enhancement of decomposition by lowered temperature (DeNeve et al., 1996). The decomposition seemingly resided at an early decomposition stage of mainly undecomposed EOC and the simulated course of EOC-induced CO_2-release appeared to converge at this stage, proposing the undecomposed EOC as potentially stable in incubation experiments. But neither conversion into microbial products (Cotrufo et al., 2013) nor physical protection (Kiem and Kogel-Knabner, 2002, Six et al., 2002) stabilised this visibly undecomposed EOC, as subsequent temperature increase enabled further decomposition. This disqualified the lowered temperature for incubation experiments, as the EOC-induced CO_2-release would be underestimated and persistence of EOC in soil therefore overestimated.

The temperature sensitivity of maize stubbles initially approached the range of Q_{10}-value 2-3, which is commonly expected for biological processes, and slightly increased, indicating that the microbial community is unable to adapt to the lowered temperature and compensate in the ongoing decomposition. As temperature sensitivity of SOC decomposition essentially depends on substrate quality, i.e. molecular size or aromaticity (Davidson and Janssens, 2006, Craine et al., 2010, Conant et al., 2011, Wagai et al., 2013), the increasing scarcity of low molecular compounds and missing activation energy for enzymatic decomposition could be the reason for increasing temperature sensitivity. In this context, leap to Q_{10}-value 6 would indicate a critical point of substrate quality change. If a specific activation energy determined this substrate quality, this point should be variable, depending on temperature-level. Contemporaneously, this point marked the maximal observed temperature sensitivity of this experiment, which was comparably low on a scale of seasonal variation in temperature sensitivity of SOC decomposition (Janssens and Pilegaard, 2003) and equally depended on the applied temperature-level (Davidson and Janssens, 2006). This complex nature of temperature sensitivity disables the temperature functions, which solely rescale the decomposition rate (DeNeve et al., 1996), to provide correct mathematical modifications of the courses of EOC-induced CO_2-release. As a consequence, a common incubation temperature remains a central issue for the comparability of incubation experiments.

The results indicated an enhancement of temperature sensitivity by a microbial community-level responses, similar to microbial community-level responses in soils of high C/N-ratios (Karhu et al., 2014). The enhancement was apparent as temperature sensitivity increased with decomposition progress and the absolute temperature effect was reversible by rewarming to a common temperature level. The concordance to microbial community-level responses in soils of high C/N is plausible, as maize stubble and plant residues in general are characterised by much higher C/N-ratios. This links temperature

sensitivity of microbial activity and nitrogen availability, and implies interaction of incubation temperature and the EOC-induced CO_2-release, limiting biochemical indicators of C_{pot} (Lashermes et al., 2009) to a specific temperature-level.

2.4.4 Influence of the SOC priming effect and C-limitation on decomposition

2.4.4.1 Priming effect

The total mineralisation of SOC over the entire incubation duration was 0.02 g (g SOC)$^{-1}$ in concordance with the average mineralisation rate of SOC in field experiments (Lashermes et al., 2009) and the equivalent average mean transit time of 50 years for SOC (Schmidt et al., 2011).

The results showed a high priming effect of wheat shoot on SOC, and therewith provided further evidence for soil organic carbon decomposition to be essentially controlled by fresh carbon availability, as EOC application induced priming (Fontaine et al., 2007). The Wheat-induced CO_2-release contained carbon of both SOC and wheat-C. The relative contribution of CO_2 from SOC in EOC-induced CO_2-release was large and could not be neglected. The priming effect therefore largely influenced C_{pot} of wheat shoot (Figure 17). This finding is in contrast to assumptions of previous investigation, which interpreted EOC-induced CO_2-release as mineralisation of EOC (Jensen et al., 2005). The soil organic carbon priming was not tested for further EOC sources, but interpretation of apparent courses of EOC-induced CO_2-release as mineralisation of EOC (Lashermes et al., 2009) remains questionable for plant residues, which constitute a major source of SOC.

The priming effect was mainly tied to the initial three to seven days of incubation. In this period of incubation, a constraint of available energy for wheat shoot and SOC mineralisation remained unlikely. (Lashermes et al., 2009) identified the EOC-induced CO_2-release in the initial 3 days of incubation as an important predictive parameter for C_{pot} estimations. Our results further imply, that this parameter could solely be replaced by biochemical parameters, if these are causally related to the priming effect.

2.4.4.2 Energy limitation of decomposition

As the rate of EOC-induced CO_2-release continuously decreased and even levelled off, before the complete initially applied carbon amount had been released, we assumed, that energy limitation for the decomposing microorganisms had been the major reason for the appearance of C_{pot} in incubation.

The application of glucose, 35 days after start of incubation was intended to reduce energy limitation for the decomposing microorganisms. The results showed glucose-induced modifications of the CO_2-release from soil columns (Figure 18), as within the first 7 days CO_2-release was drastically increased and 21 d after glucose application cumulative CO_2 release was enhanced by about 50 mg C. As the quantitative contribution of C from soil priming is presumably high, this indicates that most of the added glucose was either utilized for energy metabolism (respiration) within the first 3 weeks after application and for the microbial access of SOC.

According to the different C_{pot}-values, we expected decomposition of digestate to be more constrained by energy limitation than decomposition of wheat shoot and therewith the glucose-induced increase of CO_2 release to be larger in digestate-amended soil than in wheat shoot-amended soil. However, the glucose-induced increase of CO_2 release was very similar in soil columns without and with amendments of shoot biomass or digestate (Figure 18). The differences in cumulative CO_2 release among the three soil treatments (without amendment, amended with shoot biomass, amended with digestate) were very similar irrespective of whether glucose was applied or not applied.

Thus, the glucose-induced increase of CO_2-release in wheat shoot biomass-amended soil was somewhat higher than in digestate-amended soil or soil without amendments (Figure 19). Larger glucose-induced increase of CO_2 release in shoot biomass-amended soil may be due to (i) higher glucose-induced priming of SOC mineralisation, and/or (ii) higher glucose-induced mineralisation of shoot biomass (as compared to digestate mineralisation or SOC mineralisation), and/or (iii) higher ratio of glucose utilisation for respiration instead of growth and stabilisation in soil (Glucose added to the soil can either be absorbed by microorganisms and used for respiration and growth; glucose utilisation for growth is associated with synthesis of microbial metabolites which - at least in part – are recalcitrant. Glucose which is not taken up by microorganisms may be protected from decomposition by physical and chemical processes (Marschner et al., 2008)).

We suggest that in the soil columns which had been amended with shoot biomass 35 d before glucose application, there was a larger microbial population than in the soil columns without amendment or with digestate amendment. This suggestion is based on the assumption, that shoot biomass (primary organic biomass which was not used by microorganisms before) due to its high content of easily available organic carbon and nitrogen is a better source for microbial growth than digestate (secondary organic biomass which was remaining after prior utilisation of primary organic biomass through microorganisms). Furthermore, we suggest that the larger microbial population in soil which was previously amended with shoot biomass was suffering from energy limitation because the easily available organic carbon from shoot biomass has been largely used after 35 d. In this situation, added glucose was to a greater extent used for energy metabolism resulting in respiratory CO_2-release (iii).

In support of humification coefficients, derived from incubation experiments, the C_{pot}-values of wheat shoot and maize digestate remained roughly robust towards the glucose application (Table 7). The C_{pot} of wheat shoot was slightly decreased by the glucose application, but if this was due to and increased mineralisation of wheat shoot biomass needed to be verified.

Indeed, the results showed a slightly glucose-induced mineralisation of shoot biomass under the assumption, no glucose-induced priming of SOC occurred. As there was a little difference between the isotopic signature of soil ($\delta^{13}C$ -27 [‰]) and glucose ($\delta^{13}C$ -10 [‰]), the isotopic value of the released CO_2 would have been decreased by glucose-induced SOC-priming. This in turn would have been

detected as less wheat-shoot mineralisation than actually had occurred. In that case, the extent of the glucose-induced mineralisation was underestimated by this calculation.

As the mineralisation of wheat shoot was increased by a single glucose application, one could imagine that the continuous supply of easily available carbon compounds by the fluent soil solution in the root zone allows microorganisms to completely mineralise the wheat shoot over longer periods of time. This would imply that the measured C_{pot}-values in incubation experiments solely represent the initial mineralisation process. The C_{pot}-values, which might serve as humification coefficients in agricultural humus balancing, rather express an endpoint of mineralisation due to altered environmental conditions in incubation than a recalcitrant carbon fraction, which persists in soil due to biochemical properties (Schmidt et al., 2011).

2.4.4.3 Definition of decomposition stages for incubation experiments

Decomposition shall be further regarded as a series of three consecutive stages (Figure 21). The initial stage, lasting until the 3rd day of incubation, is probably characterised by biochemical parameters, which promote the SOC priming. With respect to a set of different plant residues (chapter 2), the former period of incubation until the 56th day of incubation is regarded as intermediate stage, characterised by biochemical parameters, which promote the mineralisation of EOC. Finally, the complete period of incubation (final stage) is characterised by parameters, which constrain mineralisation of EOC.

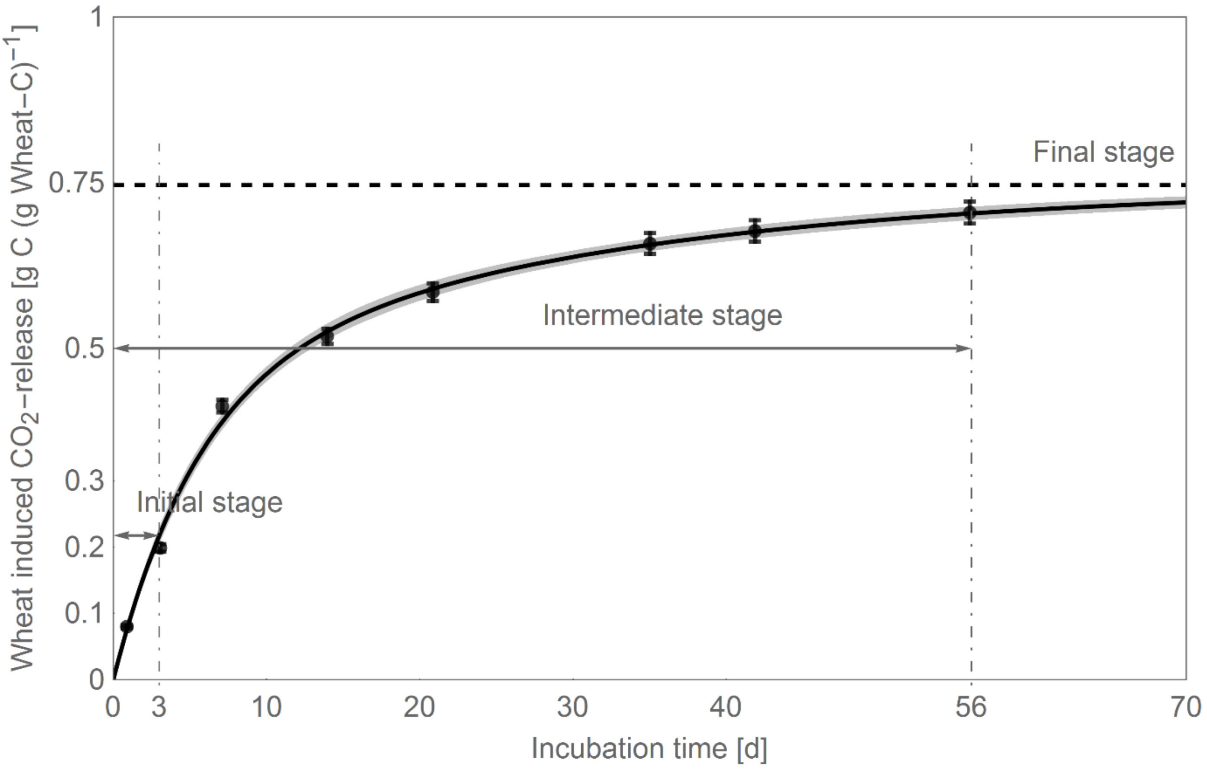

Figure 21 Wheat-induced CO_2-release (black) given as mean ± standard error (n = 4), D2 model ± 95 % CI (shaded), and limit (dashed) ± 95 % CI (dotted).

2.5 Conclusion

2.5.1 Simulation of decomposition by different mathematical model types

Decomposition of different types of plant residues, or more general EOC in different soils could be described by mathematical models, which describe the carbon loss from discrete carbon compartments at constant rate. The goodness of fit thereby depended on the number of carbon compartments considered and the order of kinetic. In this study the advance of two compartments instead of one compartment could be verified in different incubation experiments, which varied either the soil, the plant residue type or the EOC type. Furthermore, an advance of the second order decomposition kinetic instead the first order decomposition kinetic appeared. We identified the D2 model to be the most accurate. As this model provides reliable extrapolations solely after sufficient observance of EOC-induced CO2-release, we provide the prolonged incubation duration of 301 days.

All three discrete-compartment-models (D1-D3) performed the course of EOC-induced CO2-release worse under the assumption that EOC-induced CO_2-release equals EOC-addition. This implied the consideration of a fraction of EOC, which potentially resides in soil irrespective of incubation duration, indicated potential residual organic carbon (C_{pot}) as parameter for the derivation of persistence of EOC in soil.

2.5.2 Decomposition of EOC as affected by different Soils and Soil types

The soil type influenced the course of cumulative carbon loss, depending on the type of EOC initially applied. The coarse textured soil released more carbon of the applied wheat straw than fine textured soils, while several fine textured soils released most carbon of the applied digestate. Thus, the influence of the soil type on the EOC-induced CO_2-release remained low compared to the influence of EOC types. The characterisation of decomposition of different EOC types in relation to each other remains robust towards the choice of different soils for the incubation experiment.

2.5.3 Influence of incubation temperature on decomposition

The influence of a lower incubation temperature, 6 °C instead of 22 °C, revealed an enormous reduction of apparent decomposition of maize stubbles, but not enhanced persistence in soil. An enhancing response on the microbial community-level increased the temperature sensitivity of EOC-induced CO_2-release beyond the expected Q_{10}-range 2-3. As this temperature-effect was completely reversible by subsequent rewarming, low incubation temperatures would potentially undermine a large portion of EOC, which is accessible at 22 °C, failing to represent average annual conditions in a cultivated field. We therefore support the standard of 22 °C (ISO 16072) for incubation experiments.

2.5.4 Influence of the SOC priming effect and C-limitation on decomposition

In this study we assessed two aspects of energy-availability in decomposition experiments under controlled environmental conditions. Firstly, we measured the priming effect of wheat shoot to characterise the extent and duration of wheat-shoot-induced SOC mineralisation. Secondly, we proved

if decomposition of wheat shoot and maize digestate were constrained by low availability of energy-sources for microbial activity. The focus was on C_{pot}, the parameter of laboratory incubations, which shall be used for the derivation of 'humification coefficients' in agricultural humus balancing.

We found a positive priming effect for wheat shoot in the sandy loam of Berlin-Dahlem. The priming effect was essentially confined to the first 3 to 7 days of incubation, but could be verified for the complete incubation duration. The relative contribution of the priming effect to the cumulative wheat-induced CO_2-release was about 25 % and could not be neglected in the interpretation of EOC-induced CO_2-release. We here prefer the term 'decomposition' instead of 'mineralisation' of EOC. Potential residual organic carbon therefore rather represented the 'net-effect of EOC-application to soil' than the actual amount of EOC, residing in soil at the end of incubation. The confinement of the priming effect to the initial 3 to 7 days of incubation further implied for the prediction of C_{pot} that biochemical parameters, explaining the EOC-induced CO_2-release in this period of incubation should equally be related to SOC priming.

For investigation on constraints of availability of energy sources for microbial activity, we added glucose to the soil in the incubation experiment in which the soil was amended with either wheat biomass C or residues from anaerobic digestion of maize. The glucose application induced an increase in CO_2-release, which mainly occurred in the initial 7 days. This glucose-induced increase in CO_2-release was very similar in all soil treatments (no amendment, digestate amendment, or wheat shoot amendment) and C_{pot} of digestate and wheat shoot remained robust towards glucose application. There was evidence for energy-limitation to the decomposing microorganisms, as the glucose-induced increase of CO_2-release was higher in the wheat shoot-amended soil than in the digestate-amended soil. Our hypothesis, that glucose application should increase decomposition of residues from anaerobic digestion of maize for biogas production more than decomposition of straw was disproved. Therefore, we concluded that energy limitation for the decomposing microorganisms does not seriously call into question humification coefficients derived from incubation experiments. We observed a slightly increased mineralisation of wheat-shoot biomass in the initial 21 days after glucose addition. Therefore, we further conclude that energy-limitation for the decomposing microorganisms might be one criterion, which differentiates between controlled and uncontrolled environmental conditions.

3 Influence of biochemical quality on decomposition of plant residues in energy crop cultivation

3.1 Introduction

Agricultural and horticultural cropping systems in general face soil organic carbon losses through heterotrophic soil respiration, soil erosion, and leaching of dissolved organic carbon. Crop cultivation at least partly compensates for carbon losses by organic carbon input into soil via plant residues, i.e. the plant-derived organic carbon which is not harvested and removed from the field (Figure 2). Plant-derived organic carbon input includes carbon in aboveground and belowground litter from shoot organs and roots which become senescent during the vegetation period, carbon release from intact roots, and carbon in aboveground and belowground plant residues left on the field after harvest (Kuzyakov and Domanski, 2000, Jones et al., 2009, Engels, 2011).

Soil organic carbon can be conceptually divided into a fresh non-stabilized carbon pool and an older stabilized carbon pool. Plant residues deliver organic carbon to the non-stabilized carbon pool from which one part of plant residue carbon is decomposed to carbon dioxide, and the other part is transformed to more stabilized soil organic carbon (Andren and Katterer, 1997). The decomposition of plant residues is regulated by both, physicochemical soil properties (Finn et al., 2015), and the biochemical quality of plant residues (Jensen et al., 2005, Zhang et al., 2008, Amin et al., 2014, Moorhead et al., 2014). There is evidence that input of plant residues may either increase (positive priming effect) or decrease (negative priming effect) the decomposition of organic carbon from the older stabilized soil organic carbon pool (Kuzyakov et al., 2000, Shahzad et al., 2015). The priming effect is dependent on the amount and quality of plant residues (Paterson and Sim, 2013, Mazzilli et al., 2014). There is some evidence that decomposition is dependent also on anatomical tissue characteristics of the residues that influence the accessibility of tissues for microbial degradation (Lindedam et al., 2009). For example, it was suggested that in intact roots the casparian bands in the exodermis and endodermis can delay colonisation of cortex and stele tissue by decomposing microorganisms (Lindedam et al., 2009).

The amount and quality of plant residues are expected to differ between cropping systems for production of biogas (in the following text called “energy crop cultivation”) and cropping systems for food production. In energy crop cultivation the whole harvestable above-ground biomass is removed from the field. Thus, the amount of plant-derived organic carbon input into soil is expected to be lower than in cropping systems for food production, in which the above-ground biomass except the grains is incorporated into the soil. In energy crop cultivation, grain crops are often harvested prematurely, i.e. well before dead ripeness. During plant senescence many non-structural organic carbon compounds, e.g. reserve carbohydrates and amino acids, are reallocated within plants from vegetative plant organs to grains (Engels et al., 2012). In contrast, structural organic carbon compounds which are incorporated into cell walls are not reallocated. Thus, it may be expected that the biochemical quality of plant residues is dependent on the phenological stage in which plants are harvested. In accordance with this expectation

it has been found that wheat internodes harvested at physiological maturity contained less reserve carbohydrates and decomposed less than wheat internodes which were harvested at anthesis (Bertrand et al., 2009). In studies with forage plants it was found that the lignin content of plants increases with maturity (Hatfield and Fukushima, 2005). In a study with maize and wheat it was also found that lignin content increases with increasing plant developmental stage, particularly during grain filling (Abiven et al., 2011). Furthermore, also the lignin composition was modified during grain filling (Abiven et al., 2011).

Main aim of energy crop cultivation is formation of high total above-ground biomass per hectar (ha), whereas grain yield and maturity are less important. In many agro-ecological situations, this allows cultivation of special energy crop species and cultivars as well as special cropping systems including double cropping (Karpenstein-Machan, 2001, Anex et al., 2007, Baker and Griffis, 2009, Goff et al., 2010) and intercropping (Manatt et al., 2013, Voisin et al., 2014, Strickland et al., 2015). For example in Germany, double crop systems with the first crop winter rye harvested prematurely followed by warm season crops maize (*Zea mays* L.) or forage sorghum (*Sorghum bicolor* L. Moench) or sorghum sudangrass (*Sorghum bicolor* L. Moench x *Sorghum sudanense*) have been shown to outyield sole crop systems in which either winter rye or maize were cultivated (Schittenhelm, 2010). It is well documented that large differences among species (Jensen et al., 2005, Aulen et al., 2012, Redin et al., 2014) and cultivars (Machinet et al., 2011, de Graaff et al., 2013) exist with regard to chemical composition of litter and litter decomposition. Furthermore, there is evidence that the decomposition of litter mixtures from intercrops of several species can differ from that expected from separate decomposition of litter of the species composing the mixture (Gartner and Cardon, 2004). Decomposition rates of mixtures can be higher (Zeng et al., 2010, Redin et al., 2014) or lower (Liu et al., 2007) than expected from separate decomposition of the individual components. In view of the differences between energy and food crop cultivation with regard to the spectrum of crop species, cultivars and cropping system, new knowledge about species-specific, cultivar-specific and intercropping specific decomposition of plant residues is needed.

The decomposition of plant residues and the ratio of plant residues which is partitioned into the stabilized soil organic matter pool can be quantified in long-term field experiments (e.g., Poeplau et al., 2015), in litterbag studies under field (e.g., Kou et al., 2015) or controlled environmental conditional (e.g., Birouste et al., 2012) or in incubation studies under controlled environmental conditions (e.g., Redin et al., 2014). Considering the regulation of decomposition by biochemical quality of plant residues (Jensen et al., 2005, Moorhead et al., 2014), decomposition and the variability in decomposition among plant organs from different species may be predicted by various biochemical characteristics including C/N ratio (Nicolardot et al., 2001), phenol content (Grabber and Coblentz, 2009), content of various cell wall fractions (Jensen et al 2005), and lignin quality (Baldock et al., 1997, Mathers et al., 2007, Talbot et al.,

2012, Finn et al., 2015). The biochemical quality, in turn, can be measured by conventional chemical methods or by spectroscopic methods (Bruun et al., 2005, Borgen et al., 2011, Peltre et al., 2014).

In our study we addressed the following questions:

Is the decomposition of plant residues dependent on the crop species?

Is the decomposition of plant residues dependent on the type of plant residues? For addressing this question we compared decomposition of aboveground litter, aboveground harvest residue, coarse roots and fine roots i.e. plant residues remaining in soil in energy cropping systems.

Is the decomposition of plant residues dependent on plant developmental stage? For addressing this question we compared residues from winter cereals harvested either at full maturity or at flowering.

Is the decomposition of plant residues from maize, sorghum and Sudan grass dependent on whether the crops are grown as sole crop or second crop?

Does decomposition of plant residues from intercrops differ from that expected from sole crops of the species which are included in the intercrops? For addressing this question we compared residues from sole crops of oat, pea, maize and sorghum with the intercrops pea/oat and maize/sorghum.

Can the decomposition of plant residues be predicted by their chemical composition? For addressing this question we measured "van Soest"-fractions, C, N, and water-soluble carbohydrates.

We collected plant samples from field experiments which were designed to assess the effect of energy crops and cropping systems on input of plant-derived carbon into the soil. For assessment of plant residue decomposition we measured for 301 days the residue-induced CO_2 release from soil in incubation experiments under controlled environmental conditions. The course of EOC-induced CO_2-release was evaluated with a model which divides the carbon into a short-term and a long-term degradable pool, and allows the determination of the mean transit time of the biodegradable carbon pool in soil (Manzoni et al., 2012a). Our main hypothesis was that decomposition of plant residues can be predicted by their chemical composition which, in turn, is influenced by crop species, residue type, and crop management (plant developmental stage at harvest, sole vs. intercropping, sole crop vs. second crop).

3.2 Material and Methods

3.2.1 Plant residues

A field experiment was set in Berlin-Dahlem containing winter cereal (winter wheat in 2012, winter rye in 2013 and 2014), pea, oats, maize, and the new energy crops sorghum and Sudan grass in sole cropping, intercropping, and double cropping systems (Table 8) in a randomised block design with four replications (n = 4). In the cultivation years 2012-15, straw, being determined as the whole harvestable shoot above the cutting edge without the regenerative harvest organs (peas, cobs, and grain), and crop residues, containing litter, stubble, coarse root, and fine root, were collected. Litter mainly consisted of senescent leaves which were partially decayed by soil surface decomposition. Stubble represented the first shoot nodes with associated leaves below the crop cutting edge. Coarse root contained roots of

lower order, which were directly connected to the shoot and fine root described roots of higher order. Straw, stubble, coarse and fine root were sampled at annual harvest, while litter was periodically collected over the entire cultivation period. In total 180 plant residues were collected (12 crop species/ cropping systems × 5 plant residue types × 3 cultivation years), whereof 36 were straw and 144 were crop residues.

The allocation of carbon in different types of the entire crop residue was observed in a parallel investigation (see allocation coefficients, Table 9). In order to consider the continuous carbon input into soil by fine root die-back during a cultivation period, the root turnover (referred to as 'rhizodeposition') was taken into account as additional fine root input. Therefore (i) the so called 'extra root', which encompasses rhizodeposition and the excretion of mucilage, was estimated to be 0.65 times the magnitude of measured carbon in fine- and coarse roots at harvest (according to Bolinder et al., 2007), and (ii) rhizodeposition was further assumed to account for half of 'extra root'. The carbon allocation coefficients were used to calculate weighted overall means of biochemical properties for the entire crop residue of each crop species.

Table 8 Plant residues of different crops and cropping systems in energy crop cultivation, cultivation year of plant residues selected for the incubation study (n = 40), and in brackets cultivation year of plant residues selected for a sole biochemical indication of residual organic carbon (n = 20).

Cropping system	Crop species		Above-ground residue			Below-ground residue	
			Straw	Litter	Stubble	Coarse root	Fine root
Sole crop	Winter Cereal	*Triticum aestivum*	2012	2012	2012	2012	(2015)
	Pea	*Pisum sativum*	(2012)	2013	2013	2013	2013
	Oats	*Avena sativa*	(2012)	2012	2012	2012	2013
	Maize	*Zea mays*	2012	2012	2012	2012	2013
	Sorghum	*Sorghum bicolor*	(2012)	2012	2012	2012	2013
	Sudan grass	*Sorghum bicolor x sudanense*	(2012)	2012	2012	2012	2013
Intercrop	Pea-Oats	*Pisum sativum & Avena sativa*	(2012)	2012	2012	2012	(2012-15)
	Maize-Sorghum	*Zea mays & Sorghum bicolor*	(2012)	2012	2012	2012	(2012-15)
Double cropping	Winter Cereal green-cut	*Triticum aestivum*	(2012)	(2013)	2012	2012	2012
	Maize	*Zea mays*	(2012)	2012	2012	2012	2013
	Sorghum	*Sorghum bicolor*	(2012)	(2012)	(2012)	(2012)	2013
	Sudan grass	*Sorghum bicolor x sudanense*	(2012)	(2012)	(2012)	(2012)	2013

Table 9 Carbon allocation coefficients as portion of crop residue based on measurements (n = 4) in three year field-experiment in Berlin-Dahlem (Höcker et al., 2015) and on the estimation of rhizodeposition.

Cropping system	Crop species	Crop residue				
		Litter	Stubble	Coarse root	Fine root	Rhizodeposition
		Carbon allocation coefficient				
Sole crop	Winter Cereal	0.22	0.13	0.19	0.21	0.13
	Pea	0.13	0.10	0.05	0.41	0.15
	Oats	0.24	0.14	0.15	0.23	0.12
	Maize	0.04	0.33	0.23	0.15	0.12
	Sorghum	0.07	0.22	0.26	0.16	0.14
	Sudan grass	0.08	0.21	0.24	0.19	0.14
Intercrop	Pea-Oats	0.20	0.14	0.14	0.26	0.13
	Maize-Sorghum	0.04	0.24	0.27	0.16	0.14
Double cropping	Winter Cereal green-cut	0.08	0.15	0.12	0.34	0.15
	Maize	0.04	0.26	0.21	0.21	0.14
	Sorghum	0.04	0.18	0.20	0.27	0.15
	Sudan grass	0.05	0.16	0.16	0.32	0.16

Stubbles and coarse roots were cooled after harvest at 4 °C, washed and air-dried at 60 °C for at least 48 hours. Fine roots were frozen with surrounding soil, thawed, washed out and air-dried. Straw and litter were directly air-dried. All plant residues were finely milled to 1 mm particle size by cutting mills of differently sized cutting units. Straw, litter, stubbles, and coarse roots were cut by the large cutting unit of a Retsch® SM 2000 cutting mill, while fine roots were cut by the small-sized cutting unit of a Culatti® cutting mill. After milling, a pooled sample of all four field replications was formed for characterisation of biochemical quality. A subsample of each pooled sample was taken and finely ground in a Retsch® ball mill. Annually pooled fine root samples were then twice pooled to one united sample for each crop species / cropping system. The pulverised subsample was analysed for total carbon (EN 15936) and total nitrogen concentrations (EN 16168), using elementary analysis (elementar® varioMAX®) after dry combustion (Dumas, 1831). The fibre fractions hemicellulose (HEM), cellulose (CEL) and lignin (LIC) were determined for each pooled sample by their solubility in detergents (neutral detergent solution, acid detergent solution, and a concentrated sulfuric acid solution) as described for determination of plant cell wall constituents (Van Soest and Wine, 1967) and used for determination of forage digestibility (following the German standard VDLUFA, 1976). All fibre fraction determinations were conducted in a half-automatic digestion apparatus (FOSS® Fibertec™ 1020) as repeat determination with further laboratory repetitions, until two values deviated less than three per cent from their arithmetic mean. The HEM fraction was calculated as difference of neutral (NDF) and acid detergent fibre (ADF), the CEL fraction was calculated as difference between ADF and acid detergent lignin (ADL), and ADL was accounted as LIC. In addition to all fibre fractions, a soluble fraction (SOL) was calculated as difference between initial dry matter and NDF, containing both crude ash and the soluble organic matter in neutral detergent solution. Dry matter (DM) was determined at 105 °C. Furthermore, the concentration of water-soluble carbohydrates (WSC) was determined in a cold-water extract of 0.5 g plant residue dry matter in 100 ml deionised water, using the anthron method (according to Yemm and Willis, 1954, adapted by von Lengerken and Zimmermann, 1991) and spectrometrically detected in continuous flow analysis (CFA). A repeat determination of WSC was conducted with the criterion of five per cent deviation from arithmetic mean. All element levels and biochemical fractions were expressed in g (100 g DM)$^{-1}$ for characterisation of biochemical quality and in g (kg DM)$^{-1}$ for the indicator of C_{pot}.

3.2.2 Incubation experiment

3.2.2.1 Soil and Site

A sandy loam, containing 1.6 % total carbon, 0.11 % total nitrogen, and 18.5 mg (kg soil)$^{-1}$ mineral N was collected from a long-term field experiment in Berlin Dahlem with intensive farm yard manure application over the past decades. One year later, for the subsequent experiment the soil was repeatedly collected, containing 38 mg (kg soil)$^{-1}$ mineral N. Soil sampling occurred at three points along a 20 m line in the field for a pooled sample, representing the upper 30 cm of the A-horizon. The soil was sieved without drying to 2 mm particle size and stored for 10 days at 22 °C.

3.2.2.2 Setup of the incubation study

Apparent course of EOC-induced CO_2-release of 40 plant residues was measured in two incubation experiments under controlled laboratory conditions (Table 8). The second incubation experiment contained pea residues and all fine roots. In both experiments, straw was included as standard residue. This should allow comparing the results obtained by the two separate experiments. The plant residues were homogenously mixed at a rate of 400 mg EOC per 100 g soil. Then the soil was filled into small tubes (soil columns) at a bulk density of 1.1 g cm^{-3}. Soil columns with and without plant residues were prepared with 3 and 5 replications, respectively. Contrary to previous investigations (Henriksen and Breland, 1999, Jensen et al., 2005, Lashermes et al., 2009), no mineral N was added, taking limited nitrogen availability into account. Incubation temperature was 22 °C. At the start of incubation, soil water content was adjusted to 20.8 ml H_2O per 100 g soil, expressing 50 % of water holding capacity (ISO 16072). After 301 days of incubation, the mineral N concentration in each soil column was determined in an extract by spectrometric measurement (DIN 19746).

3.2.2.3 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.15 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.3 M HCl and phenolphthalein as indicator. Measurement dates were 1, 3, 7, 14, 21, 35, 56, 77, 98, 120, 162, 217, and 301 days after start of incubation. The apparent decomposition of plant residues was calculated as difference between evolved CO_2 from soil columns with and without plant residue. The course of EOC-induced CO_2-release was calculated by summing up the EOC-induced CO_2-release between two subsequent measurement dates.

As the CO_2 release was measured in two subsequent incubation experiments, there was a slight difference between the EOC-induced CO_2-release of the standard residue winter wheat straw in both experiments (compare black lines in Figure 22). For each measurement date *i*, the quotient of EOC-induced CO_2-release of the standard residue winter wheat straw in the first incubation experiment $C(t_i)_1$ and in the second incubation experiment $C(t_i)_2$ was calculated. The arithmetic mean of all quotients served as correction factor *z*:

$$z = \frac{\sum_{i=1}^{13} \frac{C(t_i)_1}{C(t_i)_2}}{13}$$

This correction factor was calculated to be 1.1, which indicated an average deviation of 10 % in the magnitude of EOC-induced CO_2-release between both experiments. For all plant residues in the second incubation experiment, each measurement of EOC-induced CO_2-release was multiplied by this correction factor to enhance comparability of decomposition in both incubation experiments (Figure 22, grey line).

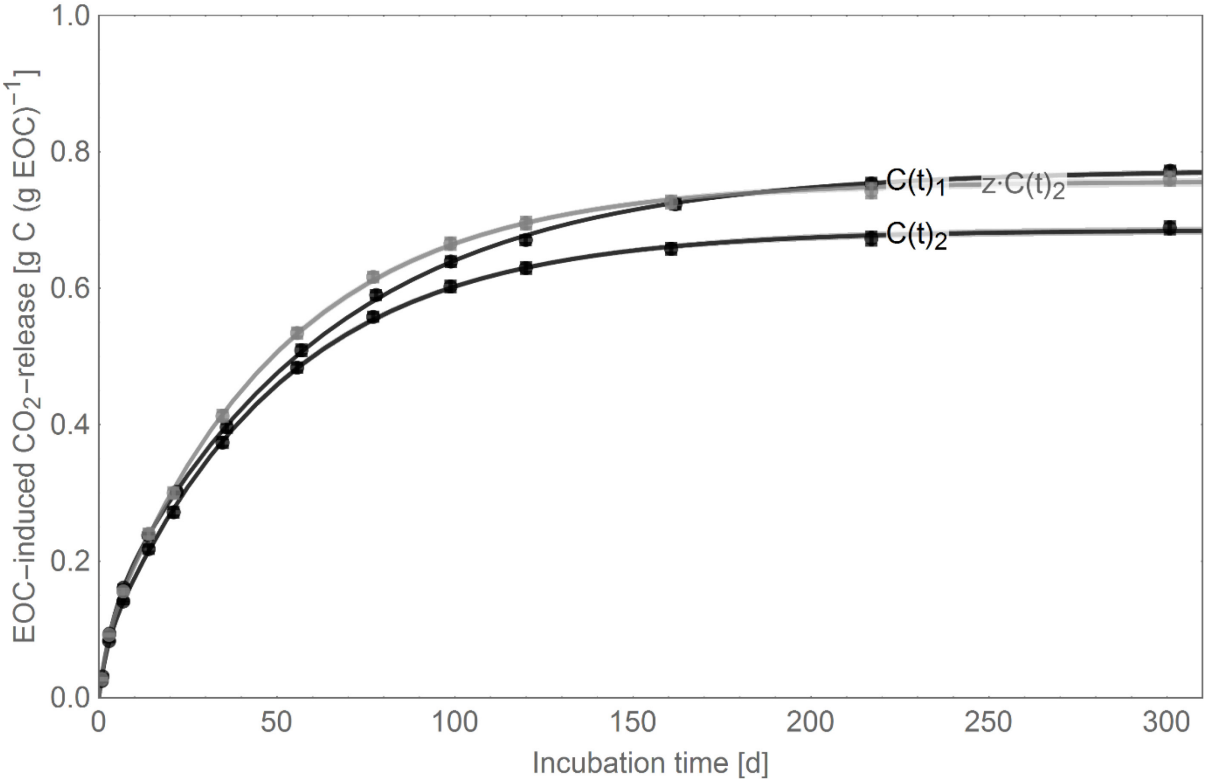

Figure 22 EOC-induced CO_2-release of the reference material wheat straw in the first (black, $C(t)_1$) and second incubation experiment (black, $C(t)_2$). Hypothetical EOC-induced CO_2-release of wheat straw in the second incubation experiment, after multiplication by the correction factor z = 1.1 (grey, $z \cdot C(t)_1$). EOC-induced CO_2-release given as mean ± standard deviation (n = 3), and D2 model ± 95% CI (shaded).

The apparent decomposition of EOC was expressed as EOC-induced CO_2-release in g C (g EOC)$^{-1}$. The EOC-induced CO_2-release from soil, which was induced by EOC application, is named "apparent" decomposition, as it integrates soil organic carbon priming and EOC mineralisation.

3.2.3 Statistical analysis and modelling

3.2.3.1 Biochemical quality

Results of elementary analysis are presented as means (least square means) of crop species / cropping systems, crop residue types, and the crop residue types of each crop species / cropping system (12 crop species / cropping systems × 4 crop residue types × 3 cultivation years (replications), N = 140). Results of the biochemical fractions WSC, HEM, CEL, and LIC are equally presented as means (least square means), but represent in case of fine roots one pooled sample of all three cultivation years (12 crop species / cropping systems × 3 crop residues (litter, stubble, coarse root) × 3 cultivation years (replications) + 12 crop species / cropping systems × 1 plant residue (fine root) × 1 pooled sample of three cultivation years, N = 113). Samples were excluded, if the carbon concentration was below 25 per cent of dry matter (outliers due to sample contamination with large amounts of adhering soil). A balanced correlation analysis of all biochemical properties was conducted, wherein values of fine roots were accounted to represent a mean of three annual values (N = 113), using the Pearson correlation

coefficient. For each biochemical parameter, a linear mixed model was set as hierarchically classified:

$$y_{ij} = \mu + a_i + b_j + c_k + ab_{ij} + \varepsilon_{ijk}$$

where a_i is the crop species / cropping system, b_j is the crop residue type, c_k is the cultivation year, and ab_{ij} is the interaction of crop species / cropping system and crop residue type. Models were set up twice, as weighted and unweighted model, under consideration of the co-variance structure and Kenward-Roger degrees of freedom approximation. Overall means of crop species / cropping system were calculated as weighted (weighting coefficients in Table 9) least square means, while overall means of crop residue type and the interaction of both were calculated as unweighted least square means. Fix effects were tested with an F-test and consecutively distinguished by pairwise comparisons, using Tukey-HSD-test (α at the 0.05 probability level). All statistical analyses were conducted in SAS® 9.2.

3.2.3.2 Potential residual organic carbon

The course of EOC-induced CO_2-release was modelled by the D2 model (details in section 2.2.5.1 on page 31). The determination of a carbon fraction remaining in soil after EOC application as portion of it effectively serving for SOC formation had been the central issue of agricultural decomposition investigation (Lashermes et al., 2009, Sleutel et al., 2005, Kirkby et al., 2013). The concept of C_{pot} in EOC identifies a carbon fraction, remaining at decomposition rate 0.02 year^{-1} by extrapolation of mathematical models, but appeared to be insensitive to lower assumptions of decomposition rate (Lashermes et al., 2009). The biodegradable carbon pool C_s was estimated by the D2 model and C_{pot} was calculated as complement in the magnitude of EOC, indicated as g C (kg EOC)$^{-1}$:

$$C_{pot} = EOC - C_s$$

3.2.3.3 Indicator I_{pot}

At first, the elaborated indicator of C_{pot} (I_{pot}) in g C (kg EOC)$^{-1}$ for a wide range of EOC samples (Lashermes et al., 2009), was validated for plant residues in energy crop cultivation (Validation). The validation criterion was the coefficient of determination R^2, calculated by the Pearson correlation coefficient r, and the adjusted determination coefficient R_a^2 which is the corrected R^2 by the number of regression variables. Secondly, the indicator was recalibrated by partial least squares regression analysis (Recalibration). The dependent regression variable was C_{pot} in g C (kg EOC)$^{-1}$, predicted by the fibre fractions *HEM, CEL,* and *LIC* in g (kg DM)$^{-1}$ and the EOC-induced CO_2-release within the first 3 days of incubation C_{3d} in g C (kg EOC)$^{-1}$ as independent regression variables. The soluble fraction (*SOL*) was not respected as predictive variable, as it resulted as complement from all fibre fractions, and therefore provided no further predictive information. Finally, the indicator was reconstituted, integrating total nitrogen concentration *N* and water-soluble carbohydrates *WSC*, both in g (kg DM)$^{-1}$, into regression (Reconstitution). As previous studies (Lashermes et al., 2009) gave evidence for normal distribution of all independent variables, normality was assumed in this study and variables were not transformed.

The multi-collinearity of the independent regression variables was revealed in previous investigations (Jensen et al., 2005, Lashermes et al., 2009) and occurred in this study as well. The partial least squares regression procedure overcomes this problem through analysing principal components (factors *F*) out of the magnitude of independent regression variables, and constituting the regression on them (Bruun et al., 2005, Lashermes et al., 2009). To select the optimal number of partial least squares components, the "leave-one-out-cross-validation" method was used. The partial least-squares regression was always run with a complete set of initial regression variables (either biochemical properties or biochemical properties and initial EOC-induced CO_2-release C_{3d}), successively leaving out variables, if they were declared to be unimportant by the Wold criterion $VIP < 0.8$ and reached coefficients < 0.2 for the regression coefficients in the centred and scaled solution.

3.3 Results

3.3.1 Biochemical quality of plant residues

For the characterisation of biochemical quality of plant residues, a water-soluble carbon fraction, being rapidly accessed by microorganisms and a less accessible carbon fraction, which mostly consists of cellulose and other cell wall constituents is being differentiated (Bertrand et al., 2009). Biochemical quality of the plant residues, which were sampled in Berlin-Dahlem in the cultivation years 2012, 2013, and 2014, was characterised by total C- and N-concentrations, the concentration of water-soluble carbohydrates (WSC), and the fibre fractions of the SCD (Van Soest and Wine, 1967). The neutral-detergent-soluble carbon fraction (SOL) in the SCD contains low-molecular compounds, i.e. low-molecular sugars, amino acids, and water-soluble phenolic compounds, but also water-soluble cell wall constituents and storage carbohydrates, such as fructan (Van Soest et al., 1991). The majority of the water-insoluble carbon fraction is being assigned to cell wall constituents and subdivided into hemicellulose (HEM), cellulose (CEL), and lignin. Thereby one should bear in mind, that the fractionation in the SCD does not exactly qualify cell wall constituents. The determined fraction of lignin therefore includes other organic compounds, which are equally insoluble in the applied sulfuric acid solution, and have been referred to as lignocellulose or 'lignin-like' (LIC) (Lashermes et al., 2009).

The biochemical quality of crop residues largely varied (Table 10). The carbon concentration in the fractionated crop residues, 38 g (100 g DM)$^{-1}$ on average, was lower than of plant materials in previous investigation (Jensen et al., 2005), whereby the both minimum and maximum values were lower. Equally, the nitrogen concentration of the crop residues was lower on average, 1 g (100 g DM)$^{-1}$, whereby solely the maximum value was lower. The crop residues on average reached C/N-ratio 49, 24 g (100 g DM)$^{-1}$ hemicellulose, 29 g (100 g DM)$^{-1}$ cellulose and 6 g (100 g DM)$^{-1}$ lignin, which was similar to previous investigation (Jensen et al., 2005). The concentration of water-soluble carbohydrates enormously varied in crop residues (Abiven et al., 2005), the coefficient of variation was 106 % as both mean and standard deviation were 10 g (100 g DM)$^{-1}$. Characteristically high concentrations of water-soluble carbohydrates were expectable in crop residues of sorghum and Sudan grass, which were

harvested long time before physiological maturity, whereas other crop species were harvested at senescence. Mean and median deviated for C/N-ratio and water-soluble carbohydrates, indicating a skewness due to more values below than above the arithmetic mean.

Table 10 Statistical measures for biochemical properties of crop residues: C total carbon, N total nitrogen, C/N-ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin. SD standard deviation, CV coefficient of variation (n = 140 for C, N, and C/N; n = 113).

Biochemical property		Statistical measures					
		Min	Max	Median	Mean	SD	CV [%]
C	[g (100 g DM)$^{-1}$]	26	45	39	38	3.6	10
N	[g (100 g DM)$^{-1}$]	0.2	2.3	0.9	1	0.4	44
C/N-ratio		18	196	40	50	30	61
WSC	[g (100 g DM)$^{-1}$]	< 1	37	4	10	10	106
HEM	[g (100 g DM)$^{-1}$]	11	40	24	24	5	21
CEL	[g (100 g DM)$^{-1}$]	17	43	29	29	6	20
LIC	[g (100 g DM)$^{-1}$]	3	18	6	7	3	39

Biochemical properties were highly correlated with each other (Table 11), which was in accordance with previous investigations (Jensen et al., 2005, Lashermes et al., 2009). These interrelations were not limited to fibre fractions in stepwise chemical digestion, but involved C/N-ratio and water-soluble carbohydrates (Jensen et al., 2005). Thus, C/N-ratio was positively correlated with water-soluble carbohydrates and cellulose. The content of water-soluble carbohydrates was negatively correlated with hemicellulose and lignin. Whereas previous investigation found a somewhat positive correlation between hemicellulose and cellulose (Jensen et al., 2005), this was not confirmed for this set of plant materials.

Table 11 Pearson correlation matrix for biochemical properties of crop residues (n = 113): C/N-ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin. Correlations in bold denote $r^2 > 0.5$; (*) significant at $p < 0.05$; (**) significant at $p < 0.01$; (***) significant at $p < 0.001$.

	Pearson correlation coefficient r				
	CN	WSC	HEM	CEL	LIC
CN	1	0.4***	-0.04	0.52***	-0.25**
WSC		1	-0.27**	0.04	-0.33***
HEM			1	-0.09	0.03
CEL				1	-0.28***
LIC					1

The biochemical quality of crop residues was dependent on crop residue type (litter, stubble, coarse root, fine root), crop species, and for specific crop species on cropping system (e.g., winter cereals harvested at full maturity or green-cut) (Table 12). The interaction of both, crop residue type and crop species/

cropping system, could be significantly identified for each biochemical property. Additionally, biochemical quality in some aspects depended on the cultivation year, as the C/N-ratio, the portion of hemicellulose, and the portion of lignin were significantly affected.

Table 12 ANOVA-Results (p-values of F-test) for the fix effects of crop species / cropping system (S), crop residue type (R), cultivation year (Y) and the interaction (C × R) on the C/N-ratio (C/N), water-soluble carbohydrates (WSC), hemicelluloses (HEM), cellulose (CEL), and lignin (LIC). P-values in bold indicate significance at the 0.05 probability level.

Measure	Effect			
	S	R	S × R	Y
C/N	**0.0001**	**< 0.0001**	**0.0055**	**< 0.0001**
WSC	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.5432
HEM	**< 0.0001**	**< 0.0001**	**0.0300**	**0.0273**
CEL	**0.0468**	**< 0.0001**	**< 0.0001**	0.5449
LIC	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0002**

The carbon-input weighted means for biochemical properties of the entire crop residue, which was composed of litter, stubble, coarse roots, and fine roots, revealed major differences between winter cereal and pea, but minor differences between C3-plants and C4-plants (Table 13). Pea differed from both winter cereal and C4-plants, as its crop residue was characterised by the lowest C/N-ratio, 23, the lowest hemicellulose portion, 16 g (100 g DM)$^{-1}$, and the highest lignification (Abiven et al., 2005), 15 g (100 g DM)$^{-1}$. The new energy crops sorghum and Sudan grass, especially in double cropping, contained huge amounts of water-soluble carbohydrates in their crop residue, 12 to 17 g (100 g DM)$^{-1}$, which was more than in crop residues of C3-plants, 1 to 4 g (100 g DM)$^{-1}$. Differences between the crop residues of C4-plants occurred in double cropping, whereby maize contained less water-soluble carbohydrates than sorghum and Sudan grass plant residues. Across all crop species/ cropping systems, the cellulose fraction remained relatively constant. Winter cereal crop residue contained the largest hemicellulose fraction, in detail 31 g (100 g DM)$^{-1}$.

Biochemical quality largely depended on the crop residue type (Table 13, mean crop residues). The C/N-ratio of fine roots (30) and litter (33) was significantly lower than of coarse roots (56) and stubble (76). Similarly fine roots and litter contained less water-soluble carbohydrates than stubbles and coarse roots, 2, 3, 16, and 12 g (100 g DM)$^{-1}$ respectively. Stubbles were thereby characterised by the highest water-soluble carbohydrate content and C/N-ratio. The proportions of hemicellulose, cellulose, and lignin finally differentiated the four types of crop residues. Fine roots contained the lowest proportion of cellulose, 21 g (100 g DM)$^{-1}$, but the highest of lignin, 11 g (100 g DM)$^{-1}$. Litter contained the lowest proportion of lignin, 6 g (100 g DM)$^{-1}$. Furthermore, fine roots contained more hemicellulose than litter, 30 and 23 g (100 g DM)$^{-1}$ respectively. Stubbles contained the highest proportion of cellulose, 33 g (100 g DM)$^{-1}$, which was higher than of coarse roots.

The crop residue of different crop species/ cropping systems differed in a few biochemical properties of litter, but in a lot of biochemical properties of stubbles and coarse roots. Litter appeared to be of a common biochemical quality, as solely differences occurred in the portions of hemicellulose and lignin. Pea litter contained the lowest portion of hemicellulose, 11 g (100 g DM)$^{-1}$, but the highest portion of lignin, 10 g (100 g DM)$^{-1}$, compared to the winter cereal, oats, or the intercrop of pea-oats. The large differences between stubbles of different crop species / cropping systems especially occurred in C/N-ratio, which was highest (154) for winter cereal and lowest (28) for pea. Stubbles of maize, sorghum and Sudan grass reached intermediate C/N-ratios of 57-73 in sole cropping and 82-95 in double cropping. Further differences occurred in the composition of fibre. Cellulose concentrations were highest for stubbles of winter cereal and lowest for stubbles of Sorghum, containing 42 and 22 g (100 g DM)$^{-1}$ cellulose, respectively. The lignification was highest for stubbles of pea, intermediate for stubbles of winter cereal, and least for stubbles of maize, containing 15, 11, and 7 g (100 g DM)$^{-1}$ lignin respectively. Stubbles of C4-plants, i.e. sorghum and Sudan grass contained 17 up to 30 g (100 g DM)$^{-1}$ water-soluble carbohydrates, which was more than of C3-plants. The lowest concentrations of water-soluble carbohydrates, 1 g (100 g DM)$^{-1}$ thereby occurred in stubbles of pea and winter cereal. The portion of hemicellulose in stubbles largely differed between pea and winter cereal, containing 16 and 29 g (100 g DM)$^{-1}$ respectively. Coarse roots could be characterised for different crop species / cropping systems similar to stubbles with the exception, that cellulose contents in coarse roots remained constant. Furthermore, stubbles and coarse roots of plants in double cropping, consecutively sharing the vegetation period, reached the highest concentrations of water-soluble carbohydrates, 17 to 31 g (100 g DM)$^{-1}$. Fine roots of different crop species / cropping systems differed in C/N-ratio, which was lowest (16) for pea and highest (35) for sorghum and Sudan grass. Furthermore, fine roots could not be distinguished in water-soluble carbohydrates and cellulose, but in hemicellulose and lignin concentrations. Winter cereal fine roots contained a large portion of hemicellulose, 40 g (100 g DM)$^{-1}$, whereas pea fine roots contained less, 29 g (100 g DM)$^{-1}$ hemicellulose. Fine roots of C3-plants contained intermediate portions of hemicellulose, 27-31 g (100 g DM)$^{-1}$. Pea fine roots were most lignified, containing 18 g (100 g DM)$^{-1}$ lignin, whereas all other crop species contained less, 7 to 11 g (100 g DM)$^{-1}$.

Table 13 Parameters for biochemical quality of the entire crop residue: C/N ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin. Means (least square means) of annually pooled samples (n = 3, cultivation years 2012-14): Different lower case letters behind means within columns (α-γ) indicate significant differences (Tukey-Test, α = 0.05) among crop species/ cropping systems; different lower case letters behind means in last column (a-c) indicate significant (Tukey-Test, α = 0.05) differences among weighted overall means for different crop species / cropping systems; different lower case letters behind means in last row (x-z) indicate significant (Tukey-Test, α = 0.05) differences among overall means for different crop residues.

Cropping system	Crop species	Crop residue																				weighted Mean (crop species / cropping system)				
		Litter					Stubble					Coarse Root					Fine Root										
		C/N	WSC	HEM	CEL	LIC	C/N	WSC	HEM	CEL	LIC	C/N	WSC	HEM	CEL	LIC	C/N	WSC	HEM	CEL	LIC	C/N	WSC	HEM	CEL	LIC
			g (100 g DM)$^{-1}$					g (100 g DM)$^{-1}$					g (100 g DM)$^{-1}$					g (100 g DM)$^{-1}$					g (100 g DM)$^{-1}$			
Sole crop	Winter Cereal	28α	3α	26α	22α	5α	154β	1α	29α	42α	11βγ	56αβ	1α	27αβ	23αβ	8α	27αβ	5α	40α	26α	7α	66a	3a	31c	28ab	8b
	Pea	26α	2α	11β	34α	10β	28α	1α	16β	33αβ	15γ	20α	2α	19αβ	27αβ	15β	19α	0α	19β	17α	18β	23b	1a	16a	28ab	15c
	Oats	36α	1α	26α	29α	4α	63αβ	6αβ	28α	38αβ	8αβ	51αβ	2α	29β	33β	8α	32αβ	1α	30αβ	20α	11α	46ab	2a	28c	30ab	8b
	Maize	27α	2α	23αβ	25α	6α	73αβ	19αβ	26αβ	33αβ	7α	75β	10αβ	25αβ	31αβ	6α	29αβ	1α	30αβ	23α	12α	51ab	8ab	26bc	27ab	7ab
	Sorghum	30α	1α	22αβ	28α	5α	57αβ	21αβ	22αβ	30β	7αβ	50αβ	21βγ	17α	21α	6α	35β	5α	31αβ	21α	11α	43ab	12bc	23bc	25ab	7ab
	Sudan grass	30α	1α	24αβ	29α	6αβ	59αβ	21αβ	24αβ	32αβ	7αβ	47αβ	22βγ	22αβ	26αβ	7α	35β	5α	27αβ	21α	11α	43ab	12bc	24bc	27ab	8b
Intercrop	Pea-Oats	39α	2α	26α	31α	5α	58αβ	10αβ	25αβ	36αβ	8αβ	51αβ	3α	28β	32αβ	8α	24αβ	1α	27αβ	20α	12αβ	43ab	4a	26bc	30b	9b
	Maize-Sorghum	34α	1α	22αβ	30α	5α	68αβ	17αβ	25αβ	34αβ	7αβ	57αβ	13αβ	17α	27αβ	5α	35β	3α	22αβ	21α	9α	49ab	8ab	21ab	28ab	7ab
Double cropping	Winter Cereal (green-cut)	25α	3α	26α	22α	4α	86αβ	21αβ	23αβ	33αβ	8αβ	48αβ	5α	27αβ	28αβ	6α	29αβ	10α	35αβ	25α	5α	47ab	10abc	28c	27ab	6a
	Maize	37α	1α	21αβ	26α	5α	95αβ	17αβ	27αβ	34αβ	6α	85β	10αβ	28β	32αβ	6α	30αβ	2α	34αβ	23α	10α	61a	8ab	28bc	29ab	7ab
	Sorghum	47α	2α	22αβ	31α	5α	92αβ	30β	23αβ	27β	5α	70β	31γ	19αβ	23αβ	6α	33β	4α	32αβ	19α	10α	61a	16c	24bc	25a	7ab
	Sudan grass	38α	1α	22αβ	30α	7αβ	82αβ	26β	23αβ	29β	5α	66αβ	30γ	22αβ	23αβ	6α	31αβ	3α	30αβ	20α	13αβ	54a	15c	24bc	26a	8b
Mean (crop residue)		33x	2x	23x	28y	6x	76z	16z	24x	33z	7y	56y	12y	23xy	27y	7y	30x	3x	30y	21x	11z					

3.3.2 Decomposition and persistence of plant residues

3.3.2.1 The course of EOC-induced CO_2-release

The selection of plant residues for incubation, containing 40 plant residues, individually straw, litter, stubble, coarse root, and fine root, represented all crop species / cropping systems and reflected the enormous variability of biochemical quality as i.e. C/N-ratio ranged from 15 to 150. Organic carbon, which is added to soil via the incorporation of plant residues, is being partially released as CO_2 and partially persists in soil for longer periods of time. In this investigation, the EOC-induced CO_2-release of plant residues was calculated as difference between CO_2 release of soil with and without the addition of plant residues. The addition of plant residues could either increase (positive priming effect) or diminish (negative priming effect) the EOC-induced CO_2-release of soil organic matter (Kuzyakov et al., 2000, Shahzad et al., 2015). The EOC-induced CO_2-release of plant residues was composed of both the priming effect and the carbon mineralisation of the plant residue.

The course of EOC-induced CO_2-release largely differed between different plant residue types. In three consecutive periods of incubation, which described decomposition stages of decreasing carbon availability, the specific differences between litter, stubble, coarse root, and fine root were proven (Figure 21). In the *initial stage*, containing soil organic matter priming, litter and stubble lost 0.13 g C (g EOC)$^{-1}$, while coarse and fine roots were more slowly decomposed. The *intermediate stage*, characterised by substantial mineralisation of EOC, differentiated the four plant residue types: Litter showed highest apparent decomposition, followed by stubble, coarse and fine roots, losing 0.60, 0.51, 0.46, and 0.37 g C (g EOC)$^{-1}$. The complete decomposition (*final stage*) included the incubation period of scarce carbon availability. Litter abruptly converged, quitting decomposition at 0.75 g C (g EOC)$^{-1}$ EOC-induced CO_2-release, wherefore a shorter period of incubation would have been sufficient. Stubble and coarse root continuously decomposed over the entire period of incubation, quitting predictably at 0.84 and 0.80 g C (g EOC)$^{-1}$ EOC-induced CO_2-release, respectively. Fine roots were the most persistent plant residues in decomposition, losing apparently 0.50 g C (g EOC)$^{-1}$. The potentially biodegradable carbon pool C_s equalled the total carbon loss in 301 days of incubation, indicating a sufficient incubation duration.

Table 14 EOC-induced CO_2-release of plant residues in different periods of incubation and the modelled (D2 model) potentially biodegradable carbon C_s, given as means: Different lower case letters behind means within rows (a-d) indicate significant differences (Tukey-Test, $\alpha = 0.05$) among crop residues

Decomposition stage (period of incubation)	Crop residue			
	Litter	Stubble	Coarse root	Fine root
	EOC-induced CO_2-release [g C (g EOC)$^{-1}$]			
Initial (3 d)	0.13a	0.13a	0.11b	0.06c
Intermediate (56 d)	0.60a	0.51b	0.46c	0.37d
Final (301 d)	0.75a	0.82b	0.78c	0.50d
C_s (∞)	0.75	0.84	0.80	0.49

The course of EOC-induced CO_2-release and the total carbon loss of plant residues significantly differed between different crop species, and for some crop species between different cropping systems, i.e. between winter wheat as sole crop and green-cut winter wheat in double cropping (Figure 23, Figure 24). The parallel first-order decomposition kinetic of the D2 model very well fitted to the observed course of EOC-induced CO_2-release in the entire period of incubation.

C3-plant cultivation across the magnitude of cropping systems appeared to come up with highly variable course of EOC-induced CO_2-release of litter, stubble, coarse root, and fine root, as both botanical family and cultivation period changed (Figure 23). Pea, representing legumes, contrasted cereals, as stubble, coarse and fine root were extremely stable, but litter highly decomposable. The stability of pea stubble and coarse root was an issue of the final decomposition stage, as both early turned into convergence, whereas the stability of fine root issued all decomposition stages. Beyond these contrasting differences of pea and cereals, minor differences occurred between oats and winter wheat, representing summer and winter cereal respectively. After similar decomposition in the initial three days of incubation, oats litter lost less carbon than winter wheat litter in subsequent periods of incubation, whereas oats stubbles lost more carbon in the first 56 days of incubation than winter wheat stubbles and then abruptly converged. The coarse roots of oats lost more carbon than coarse roots of winter wheat from the beginning on. In the intercropping system of pea and oats, the crop residue was obviously dominated by oats and therefore decomposed like the crop residue of the sole crop oats. In the double cropping system, the crop residues of green-cut winter wheat lost more carbon than crop residues of winter wheat in sole cropping from the beginning on. Green-cut stubble and fine roots appeared with enormous EOC-induced CO_2-release in the initial three days of incubation decomposition, which was apparently higher than of winter cereal, whereas stubbles of winter wheat in both cropping systems similarly decomposed in the first 56 days of incubation.

In C4-plant cultivation, crops and cropping systems slightly varied in course of EOC-induced CO_2-release of litter and stubble (aboveground residues), but substantially in course of EOC-induced CO_2-

release of coarse and fine root (belowground residues) (Figure 24). Compared to C3-plants, litter was more stable, whereas stubble, coarse root, and fine root were less stable in decomposition. Contrary to C3-plants, differences between crop species / cropping systems predominantly occurred in the beginning and the end of incubation. Stubble and coarse root of the new energy crops sorghum and Sudan grass lost large amounts of carbon in the first three days of incubation, providing evidence for positive soil organic matter priming. Subsequently, slight differences occurred between crop species / cropping systems in decomposition of litter and stubble, but considerable differences in decomposition of coarse root and fine root. Stubble and coarse roots of sorghum und Sudan grass lost the highest amounts of total carbon in decomposition. Both the crop species and the cropping system of C4-plants influenced course of EOC-induced CO_2-release of belowground residues. In maize-sorghum intercropping, the course of EOC-induced CO_2-release for coarse root finally converged, appearing to be persistent in laboratory incubation, whereas decomposition of maize and sorghum coarse roots in sole cropping continued in the entire incubation period. Double cropping systems, which cultivated two crop species in serial for one vegetation period, differed in another important aspect from sole cropping. While the aboveground residues of maize were less stable in double cropping, coarse root equally decomposed, and inversely, fine roots of maize, sorghum and Sudan grass were more stable than in sole cropping.

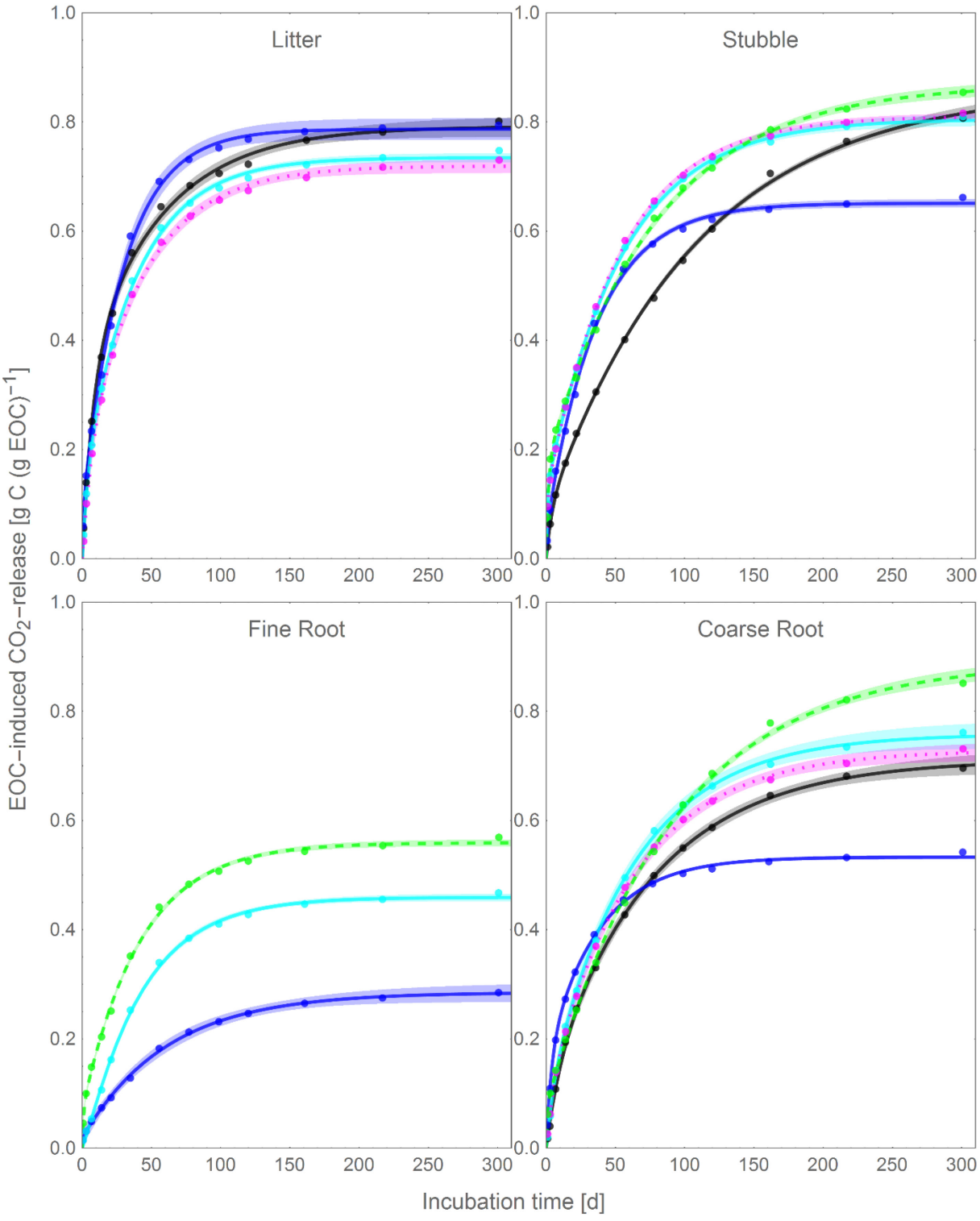

Figure 23 Decomposition in C3-plant cultivation: Winter wheat (black), Green-cut winter wheat (green dashed), pea (blue), oats (cyan), and pea-oats intercropping (magenta dotted). EOC-induced CO_2-release given as mean (n = 3), and D2 model ± 95% CI (shaded). EOC Exogenous organic carbon.

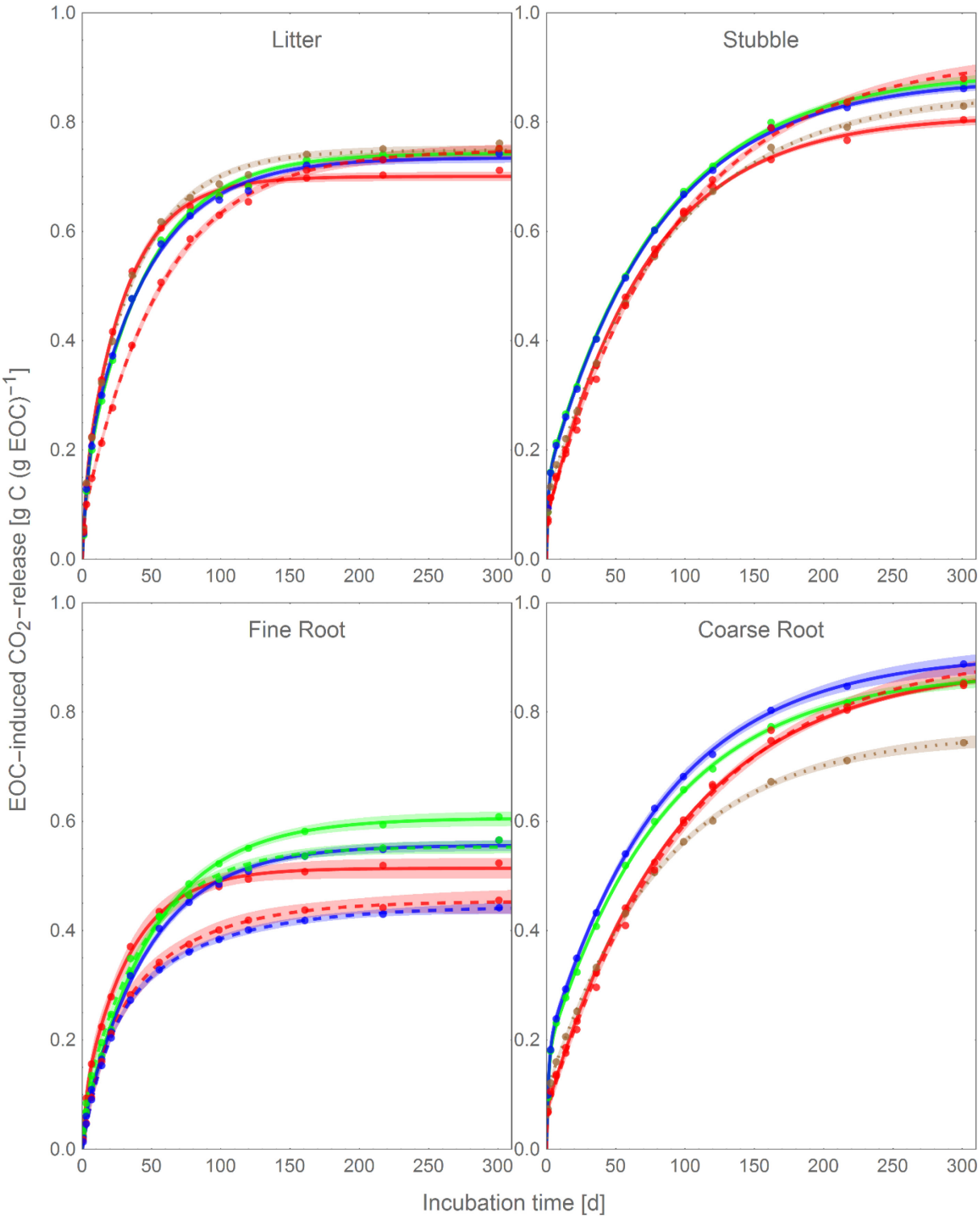

Figure 24 Decomposition in C4-plant cultivation: Maize (red), sorghum (green), Sudan grass (blue), maize-sorghum intercropping (brown dotted), maize double cropping (red dashed), sorghum double cropping (green dashed), Sudan grass double cropping (blue dashed). EOC-induced CO_2-release given as mean (n = 3), and D2 model ± 95% CI (shaded). EOC Exogenous organic carbon.

3.3.2.2 C_{pot}

The calculated C_{pot}-values ranged between 78 and 775 g C per kg EOC, whereby for all crop species the C_{pot}-values of fine roots were higher than of other plant residues (Table 15). The highest C_{pot} was obtained for fine roots of pea, 715 g C (kg EOC)$^{-1}$, which largely differed from those of the grass family, ranging to 394 g C (kg EOC)$^{-1}$ for sorghum in sole cropping. The C_{pot} of litter was somewhat higher than C_{pot} of stubbles and coarse roots for most of the crop species, exclusive pea and winter wheat. In contrast to other plant residues, the litter was less affected by crop species or cropping system and ranged from 208 to 300 g C (kg EOC)$^{-1}$. The C_{pot}-values of stubbles ranged from 78 g C (kg EOC)$^{-1}$ for maize in double cropping to 349 g C (kg EOC)$^{-1}$ for pea. The C_{pot}-values of coarse roots similarly ranged from 85 g C (kg EOC)$^{-1}$ for maize in sole cropping to 467 g C (kg EOC)$^{-1}$ for pea. Winter wheat straw left 208 g C (kg EOC)$^{-1}$ in soil.

The crop residues of the new energy crops sorghum and Sudan grass reached lower C_{pot}-values than maize, i.e. litter of maize left 300 g C (kg EOC)$^{-1}$, while litter of sorghum and Sudan grass left 257 and 265 g C (kg EOC)$^{-1}$ in soil. The C_{pot}-values of the C4-plants further depended on the cropping system. For maize, a higher C_{pot} of litter occurred in sole cropping than in double cropping, 187 and 87 g C (kg EOC)$^{-1}$ respectively. In contrast, the C_{pot}-values of fine roots were higher in double than in sole cropping for all of the C4-plants. For winter cereals, the C_{pot} of coarse roots depended on the stage of maturity at harvest, which accorded to previous investigation (Bertrand et al., 2009). Furthermore, the C_{pot} of plant residues in intercropping systems differed from C_{pot}-values of both involved crop species in sole cropping, i.e. C_{pot} of coarse roots in the intercropping of maize-sorghum, 238 g C (kg EOC)$^{-1}$ was significantly higher than in the sole cropping of maize or sorghum, 112 and 126 g C (kg EOC)$^{-1}$ respectively. In contrast to this, C_{pot} of coarse roots in the intercropping system of pea and oats, 271 g C (kg EOC)$^{-1}$, was substantially lower than the arithmetic mean of pea or oats in sole cropping, 476 and 242 g C (kg EOC)$^{-1}$ respectively. Such non-additive patterns in crop residue decomposition of different crop species have been found in previous investigation (Gartner and Cardon, 2004).

Table 15 Potential residual organic carbon (C_{pot}) in soil after decomposition of plant residue. Calculation with D2 model as mean ± 95% CI

Cropping system	Crop species	Shoot residue			Root residue	
		Straw	Litter	Stubble	Coarse root	Fine root
		C_{pot} [g C (kg EOC)$^{-1}$]				
Sole crop	Winter Cereal	226 ± 5	208 ± 19	137 ± 21	288 ± 24	
	Pea		213 ± 20	349 ± 8	467 ± 4	715 ± 18
	Oats		265 ± 9	195 ± 11	242 ± 26	541 ± 7
	Maize	191 ± 13	300 ± 8	187 ± 10	112 ± 9	486 ± 19
	Sorghum		257 ± 8	107 ± 13	126 ± 16	394 ± 14
	Sudan grass		265 ± 9	119 ± 10	94 ± 24	444 ± 10
Intercrop	Pea-Oats		281 ± 13	189 ± 7	271 ± 18	
	Maize-Sorghum		251 ± 9	145 ± 10	238 ± 15	
Double cropping	Winter Cereal green-cut			130 ± 14	108 ± 17	441 ± 6
	Maize		251 ± 14	78 ± 19	85 ± 32	546 ± 25
	Sorghum					447 ± 12
	Sudan grass					556 ± 15

3.3.2.3 Influence of biochemical quality on the course of EOC-induced CO_2-release and C_{pot}

Relations between EOC-induced CO_2-release and biochemical properties of initial EOC could be determined throughout all decomposition stages (Table 16). Initially, water-soluble carbohydrates and cellulose strongly enhanced apparent decomposition, while hemicellulose and lignin stated a hindrance, being in conformance with previous investigation (Jensen et al., 2005). Controversially, C/N-ratios were positively correlated to this excessive initial CO_2-evolution, indicating the emergent importance of easily available carbon for microbial activity. Intermediately (56 days of incubation), a relation between carbon decomposition and biochemical quality parameters disappeared except for lignin and the derived quotient of lignin to nitrogen concentration, indicating consumed nitrogen resources though a breakdown of lignin (referred to as microbial mining in Craine et al., 2007) and therewith providing support for an emergent need of external nitrogen for microbial decomposition. Finally, C/N-ratio and cellulose were positively correlated to apparent decomposition, as carbon was becoming scarce again. This explained the negative correlation of lignin to apparent decomposition as energy scarcity in contrast to recalcitrance. Mineral N concentrations in soil columns with and without plant residues finally added up to 73 ± 30 respectively 92 ± 46 mg $(\text{kg soil})^{-1}$ on average, indicating sufficient N mobilisation through SOC decomposition. In spite of initial nitrogen in plant residues being related to less EOC-induced CO_2-release, the magnitude of decomposition was mainly influenced by carbon availability, as nitrogen scarcity in final decomposition could be excluded. Lastly, decomposition stages themselves were interrelated, revealing a strong relation between the initial and final decomposition stage with emerging importance for the prediction of C_{pot} (Lashermes et al., 2009).

There were several relations of biochemical quality, course of EOC-induced CO_2-release, and C_{pot} of plant residues (Table 16, last row). The EOC-induced CO_2-release in the initial three days of incubation was negatively correlated with C_{pot} (Lashermes et al., 2009). Numerous significant correlations of biochemical properties to C_{pot} occurred. Lignin was positively correlated, whereas more water-soluble carbohydrates and more easily accessible cell wall constituents such as cellulose were negatively correlated to C_{pot}. Furthermore, a negative correlation of lignin/nitrogen-ratio to C_{pot}-values occurred, indicating the less breakdown of highly lignified plant residues in the scarcity of easily available N compounds.

Table 16 Pearson correlation coefficients of EOC-induced CO_2-release C (t_i) and biochemical quality of plant residues (n = 40). C/N-ratio, N total nitrogen concentration, LIC/N lignin to nitrogen ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin, C_{pot} Potential residual organic carbon. Correlations in bold denote $r^2 > 0.5$; (*) significant at $p < 0.05$; (**) significant at $p < 0.01$; (***) significant at $p < 0.001$.

	Pearson correlation coefficient (*r*)									
EOC decomposition				Biochemical quality indices				Stepwise chemical digestion		
	C_{3d}	C_{56d}	C_{120d}	C/N	N	LIC/N	WSC	HEM	CEL	LIC
Initial decomposition stage of the potentially biodegradable carbon pool C_s										
C_{1d}				0.51***	-0.52***	0.15	**0.78*** **	-0.42**	0.36*	-0.54***
C_{3d}	**1**			0.36*	-0.36*	-0.03	0.67***	-0.44**	0.27	-0.58***
Intermediate decomposition stage of potentially biodegradable carbon pool C_s										
C_{7d}	**0.92*** **			0.14	-0.18	-0.22	0.41**	-0.34*	0.24	-0.55***
C_{14d}	**0.78*** **			-0.05	-0.04	-0.38*	0.18	-0.23	0.20	-0.53***
C_{21d}	**0.71*** **			-0.11	-0.01	-0.43**	0.08	-0.18	0.21	-0.53***
C_{35d}	0.68***			-0.13	-0.01	-0.46**	0.04	-0.18	0.24	-0.54***
C_{56d}	**0.73*** **	**1**		0.01	-0.17	-0.36*	0.14	-0.20	0.36*	-0.63***
Final decomposition stage of the potentially biodegradable carbon pool C_s										
C_{77d}	**0.76*** **	**0.98*** **		0.19	-0.36*	-0.22	0.26	-0.20	0.46**	-0.71***
C_{120d}	**0.76*** **	**0.86*** **	**1**	0.45**	-0.60***	0.04	0.42**	-0.20	0.58***	**-0.76*** **
C_{217d}	**0.72*** **	0.70***	**0.96*** **	0.64***	**-0.76*** **	0.25	0.52***	-0.21	0.61***	**-0.75*** **
C_{301d}	**0.71*** **	0.65***	**0.94*** **	0.68***	**-0.79*** **	0.30	0.54***	-0.21	0.62***	**-0.74*** **
Long-term biodegradable carbon pool *1-*C_s										
C_{pot}	-0.67***	-0.57***	**-0.90*** **	**-0.73*** **	**0.82*** **	-0.38*	-0.56***	0.21	-0.62***	**0.72*** **

3.3.2.4 Biochemical indication of C_{pot} (Indicator I_{pot})

Based on the significant relation between biochemical quality, course of EOC-induced CO_2-release and C_{pot} of EOC in soil, current indicators (Lashermes et al., 2009) of C_{pot} could be validated, recalibrated and reconstituted (Table 17). Indicators were disposed for the D2 and D3 model, providing C_{pot} estimations. Partial least squares regressions were performed indicating C_{pot} by (A) biochemical quality in terms of stepwise chemical digestion and by (B) biochemical quality and initial course of EOC-induced CO_2-release. In spite of integrating the neutral-detergent soluble fraction (SOL) into regression, this study included hemicelluloses (HEM) and therefore all fibre fractions of stepwise chemical digestion in the regression variables.

The current regressions, which had been set up in previous investigation (Lashermes et al., 2009) were validated with the biochemical quality and decomposition data of the 40 incubated plant residues, recalibrated, and reconstituted (Figure 25). The validation of predicted C_{pot} -estimations for the 40 plant residues by the current regressions verified a significant positive relation to actually calculated C_{pot}-values with the D2 model, but equally a lack of sensitivity, as low C_{pot}-values were overestimated and high C_{pot}-values were underestimated. This was more obvious for the regression types based on biochemical quality (A) than for the regressions based on biochemical quality and initial course of EOC-induced CO_2-release (B). Moreover, the coefficients of determination, 0.49 (A) and 0.67 (B) were relatively low. This motivated a recalibration of the regressions, which was conducted as partial least squares regression analysis (analogous to Lashermes et al., 2009). The partial least squares regression identifies main components (factors F) out of the magnitude of regression variables and relates these factors to the dependent regression variable. The recalibrated regressions were based on less components (factors F) than the current ones. The recalibration of these indicators enhanced the coefficients of determination to 70 (A) and 75 (B), delivering more adequate predictions, especially for the regression type (A). The recalibration thereby emphasized lignin contents with a higher regression coefficient and turned the influence of cellulose in regression (B) from a positive into a negative coefficient. A further reconstitution of the current indicators by the inclusion of the total N and water-soluble carbohydrate concentrations could further improve the coefficients of determination to 0.84 (A) and 0.89 (B). The inclusion of the water-soluble carbohydrate contents became obsolete (Wold VIP < 0.8) in regression type B, which already contained the EOC-induced CO_2-release in the initial three days of incubation as regression variable. According to the coefficient of determination, this regression was finally proposed as I_{pot}, considering N concentration on account of partially nitrogen limited decomposition:

$$I_{pot} = 338 + 18\,N - 0.5\,CEL + 0.8\,LIC - 1.2\,C_{3d}$$

where I_{pot} is the portion of the EOC contributing to soil organic carbon replacement in crop agro-ecosystems, N [g N (kg DM)$^{-1}$] is the total N concentration of the plant residue, CEL and LIC [g (kg DM)$^{-1}$] are the cellulose and lignin fractions and C_{3d} [g C (kg EOC)$^{-1}$] is the EOC-induced CO_2-release in the initial three days of incubation.

Table 17 Validation, recalibration, and reconstitution of current partial least squares (PLS) regressions predicting residual organic carbon (C_{pot}) in residues of energy crop cultivation [g C (kg EOC)$^{-1}$] by (A) biochemical quality of residues or (B) biochemical quality and initial apparent decomposition of residues. Results of correlations used for validation of previous regressions and of the cross-validation method used for calibration of regressions: R^2 Coefficient of determination, R_a^2 adjusted determination coefficient, F number of factors in PLS regression. All regressions were significant at the 0.001 probability level. N total nitrogen concentration [g N (kg DM)$^{-1}$], WSC water-soluble carbohydrates [g (kg DM)$^{-1}$], SOL neutral-detergent-soluble matter [g (kg DM)$^{-1}$], CEL cellulose [g (kg DM)$^{-1}$], LIC lignin [g (kg DM)$^{-1}$], C_{3d} apparent decomposition in 3 days of incubation at 22 °C [g C (kg EOC)$^{-1}$]

Model for C_{pot} estimation		Variables in the regression	Coefficients					Validation / Calibration (n = 40)		
			a0	a1	a2	a3	a4	R^2	R_a^2	F
Validation of current indicators of C_{pot} (Lashermes et al., 2009) for residues of energy crop cultivation										
A	D2	SOL, LIC	226	0.2	1.2			0.49	0.48	2
B	D2	SOL, CEL, LIC, C_{3d}	445	0.5	-0.2	0.7	-2.3	0.67	0.64	3
Recalibration of current indicators of C_{pot} (Lashermes et al., 2009) for residues of energy crop cultivation										
A	D2	HEM, CEL, LIC	302	0.4	-1.1	2.5		0.70	0.68	1
B	D2	CEL, LIC, C_{3d}	523	-0.9	1.9	-1.4		0.75	0.74	1
Reconstitution of current indicators of C_{pot} (Lashermes et al., 2009) for residues of energy crop cultivation										
A	D2	N, WSC, CEL, LIC	269	13	-0.5	-0.7	1.5	0.84	0.83	1
B	D2	N, CEL, LIC, C_{3d}	338	18	-0.5	0.8	-1.2	0.89	0.88	3

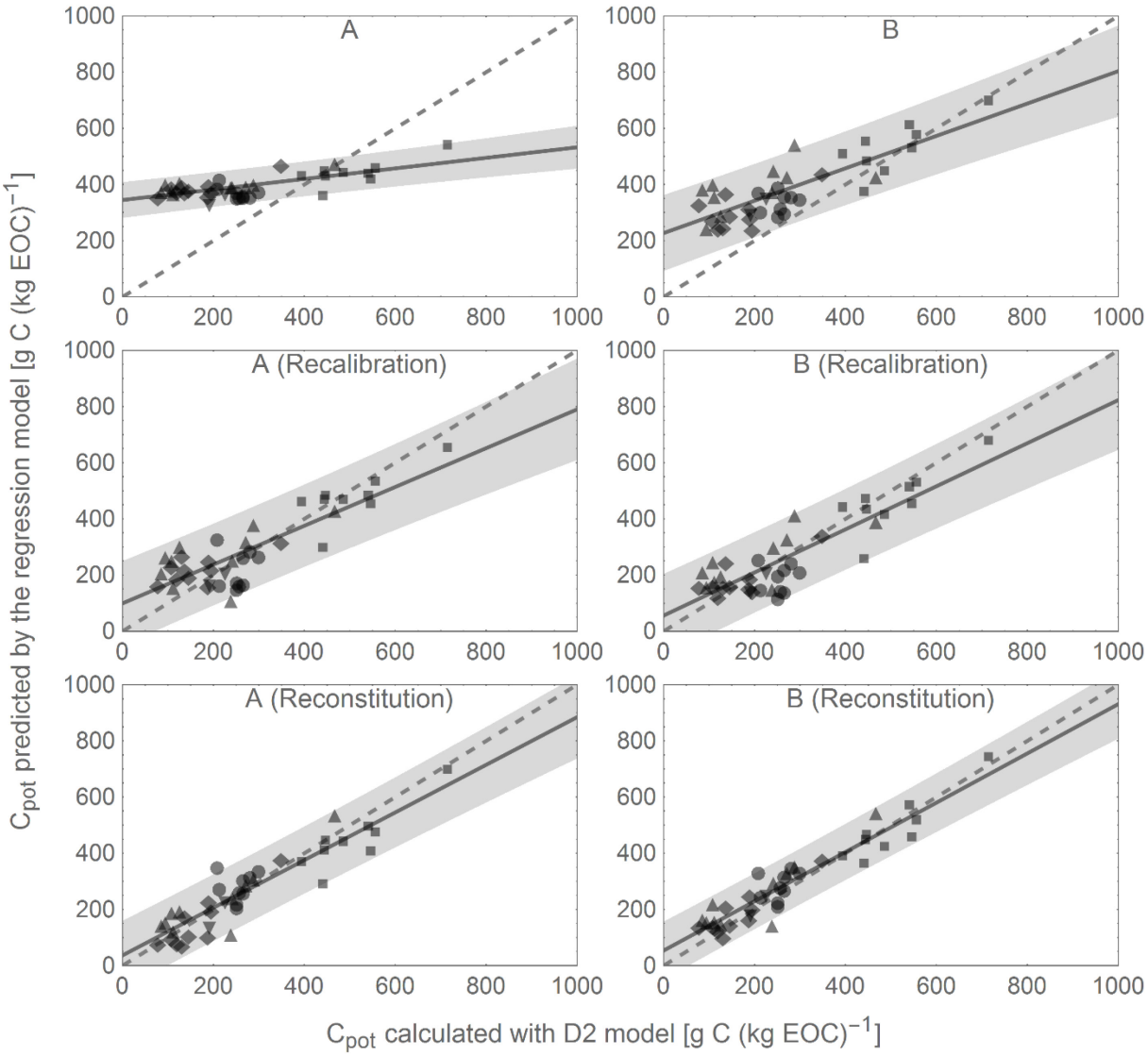

Figure 25 Potential residual organic carbon (C_{pot}) calculated with D2 model versus current indicators: (A) 226 + 0.2 SOL + 1.2 LIC and (B) 445 + 0.5 SOL – 0.2 CEL + 0.7 LIC – 2.3 C_{3d}, their recalibrations: (A) 302 + 0.4 HEM – 1.1 CEL + 2.5 LIC, and (B) 523 – 0.9 CEL + 1.9 LIC – 1.4 C_{3d}, and their reconstitutions: (A) 269 + 13 N – 0.5 WSC – 0.7 CEL + 1.5 LIC, and (B) 338 + 18 N – 0.5 WSC + 0.8 CEL – 1.2 C_{3d}. Relation given as linear regression (black line) ± 0.95 % PI (shaded) towards the background of the 1:1-relation (dashed gray line). Different symbols indicate different types of plant residues (n = 40): Straw (▼), litter (●), stubbles (◆), coarse roots (▲), and fine roots (■).

The biochemical indication of C_{pot}-values with the reconstituted regression type B was conducted for plant residues of each crop / cropping system for a differentiated evaluation (Table 18). The biochemically indicated values for fine roots underestimated apparent C_{pot}-values, especially for green-cut cereal, maize and Sudan grass in double cropping, whereas for coarse roots apparent C_{pot}-values were mostly overestimated. Intercropping maize-sorghum coarse roots were uniquely underestimated by 125 g C (kg EOC)$^{-1}$, providing evidence for a stabilisation effect, which could not be predicted by the applied parameters of biochemical quality. Litter and stubble were partially over- or underestimated, as ripening and senescence varied.

Table 18 Indication of C_{pot} in plant residues by reconstituted regression (A) 269 + 13 N – 0.5 WSC – 0.7 CEL + 1.5 LIC. Estimates ± difference to calculated C_{pot}-value. EOC Exogenous organic carbon.

Cropping system	Crop species	Shoot residue						Root residue			
		Straw		Litter		Stubble		Coarse root		Fine root	
		C_{pot} [g C (kg EOC)$^{-1}$]									
Sole crop	Winter Cereal	228	– 2	351	– 143	176	– 39	310	– 22	330	
	Pea	293		274	– 61	379	– 30	539	– 72	697	+ 18
	Oats	212		306	– 40	197	– 2	244	– 3	494	+ 46
	Maize	138	+ 54	338	– 38	104	+ 84	125	– 13	439	+ 47
	Sorghum	144		262	– 5	98	+ 9	197	– 71	367	+ 27
	Sudan grass	180		260	+ 5	80	+ 39	156	– 62	408	+ 35
Intercrop	Pea-Oats	254		316	– 36	229	– 40	290	– 18	527	
	Maize-Sorghum	147		220	+ 31	107	+ 38	114	+ 125	356	
Double cropping	Green cut winter cereal	230		317		72	+ 59	191	– 83	288	+ 153
	Maize	116		208	+ 44	79	– 1	145	– 60	405	+ 142
	Sorghum	164		187		58		116		443	+ 3
	Sudan grass	155		182		72		118		472	+ 84

3.4 Discussion

3.4.1 Influence of crop species and cropping system on biochemical quality of crop residues

The results showed a significant influence of the crop residue type, crop species and cropping system on the biochemical quality of crop residues (Table 12, Table 13). The significant differences between biochemical properties of different plant residue types confirmed the fractionation of the cultivated plant into straw, litter, stubbles, and fine roots as a suitable one for cropping systems. The C/N-ratio, the portion of hemicellulose, and the portion of lignin were further influenced by the cultivation year (Table 12). This confirmed the necessity of at least 3 cultivation years in field experiments for the biochemical characterisation of plant residues. Although large annual variability of biochemical properties limited the comparability of different crop species and cropping systems, the crop residue types stubble and coarse root significantly differed among crop species and cropping systems. As stubble and coarse root

represented large proportions of the entire crop residue, especially for C4-plants, they biochemically characterised the cultivation-specific return of crop residues to the soil.

The carbon concentration was 38 % of dry matter on average of all collected crop residues, but varied between 26 % and 45 % of dry matter (Table 10). Tao et al. (2012) showed differences in the carbon concentration between crop species, whereas Thomas and Martin (2012) further showed differences between different plant tissues. However, carbon concentrations below 40 % of dry matter might rather be artefacts due to EOC-induced CO_2-release after sampling or due to adhering inorganic mud (e. g. soil particles) at crop residues. EOC-induced CO_2-release after sampling might have occurred via respiration, leaching of soluble organic carbon during the sample preparation (e.g. the separation of roots from the soil) and via losses of volatile carbon compounds during the air-drying of plant residue samples (Thomas and Martin, 2012). The nitrogen concentration and likewise the C/N-ration varied much more than the carbon concentration between different crop residues. The C/N-ratio better expressed the amount of nitrogen in plant tissues than the N concentration, as it is not affected by adhering inorganic mud. The C/N-ratio decreased from stubble and coarse root to litter and fine root, which indicated higher nitrogen concentrations in litter and fine root than in stubble and coarse root. Maize, Sorghum and Sudan grass were characterised by the highest C/N-ratio in the total crop residue, whereas the legume pea was characterised by the lowest C/N-ratio (Table 13). As nitrogen in plant residues plays an important role for efficient microbial carbon use (Manzoni et al., 2012b) and the formation of stable organic matter (Cotrufo et al., 2013), the higher N concentrations in pea, fine roots, and litter implied an enhancement of both.

The evaluation of the C4-plants maize, sorghum, and Sudan grass towards the background of established C3-plants revealed similarities in various biochemical properties. For each biochemical property, C3-plants varied in a broad range (Jensen et al., 2005), due to botanical family (grass family, legumes), different cultivation periods (summer-annual, winter-annual), and different ripening (green-cut, mature winter cereal), so that C4-plants irrespective of cropping system solely differed from C3-plants as they contained higher amounts of water-soluble carbohydrates in the crop residue. The concentration of water-soluble carbohydrates largely varied from 0.5 % of dry matter in fine roots of pea to 30 % of dry matter in stubbles of Sorghum in double cropping (Table 13). Large differences in water-soluble carbohydrate concentrations of different plant residues have been reported before (Abiven et al., 2005, Jensen et al., 2005). Across all crop species, the concentration of water-soluble carbohydrates was higher in stubbles and coarse roots than in litter and fine roots. Crop species of the grass family are able to store large amounts of carbohydrates in vegetative plant organs before flowering (Engels et al., 2012). These carbohydrates can be mobilised in later growth stages and transported into the grains. Therefore, the water-soluble carbohydrate concentrations were especially high in stubbles and coarse roots of C4-plants, which were harvested before ripeness and senescence, and the green-cut cereals, because the translocation from vegetative to generative plant organs had not been completed.

The concentrations of hemicellulose varied from 11 % of dry matter in pea litter to 40 % of dry matter in fine roots of winter cereal (Table 13), which was a similar range as previous investigations had reported for plant residues before (Abiven et al., 2005, Jensen et al., 2005). The influence of the crop species on the concentration of hemicellulose was comparably low. Hemicelluloses, such as glucans and xylan are essential cell-wall-constituents, coating cellulose fibrils (Strasburger et al., 2014). The highest hemicellulose concentrations were observed in fine roots, for a self-evident reason. Several polysaccharides in the root hair excretions (mucilage) have been assigned to the fraction of hemicellulose (Rasse et al., 2005).

The concentrations of cellulose in plant residues varied from 17 % of dry matter in fine roots of pea to 43 % of dry matter in stubbles of winter cereal (Table 13), and were in a common range with previous investigations on plant residues (Abiven et al., 2005, Jensen et al., 2005). On average of all crop species, cellulose concentrations were lowest for fine roots (21 % of dry matter), and highest for stubbles (33 % of dry matter). Stubbles carry the whole shoot, and are necessarily equipped with the highest density of cellulose fibre, whereas fine roots are more exposed to compressive forces between soil particles than tensile forces of the whipping shoot.

The lignin concentrations varied from 3.9 % of dry matter in litter of green-cut cereals to 18.4 % of dry matter in fine roots of pea. (Table 13), and were in a common range with previous investigations on plant residues (Abiven et al., 2005, Jensen et al., 2005). On average of all crop species, the lignin concentrations were higher in fine roots (10.9 % of dry matter) than in stubbles (7.6 % of dry matter) and coarse roots (7.6 % of dry matter). Other investigations, which did not distinguish between coarse- and fine roots revealed likewise higher lignin concentrations in roots than in above-ground residues (Abiven et al., 2005). Large differences occurred between the total crop residues of different crop species, as for the legume pea, in which lignin concentrations were higher than in any other crop species of the grass family. Abiven et al. (2005) likewise identified the highest lignin concentrations in roots, stems, and leaves of legumes.

3.4.2 Influence of crop species and cropping system on decomposition of plant residues

The kinetic of CO_2-evolution and the totally evolved amount of carbon was significantly different between different plant residue types and crop species (Figure 23, Figure 24). On average of all crop species, the addition of litter caused the highest EOC-induced CO_2-release in the initial 36 days of incubation, but in subsequent incubation time the EOC-induced CO_2-release largely decreased, resulting in even less total carbon loss than of stubbles and coarse roots. The course of EOC-induced CO_2-release and total carbon loss of stubbles and coarse roots was similar. The total carbon loss of fine roots was lowest. On average of all crop residues, the total carbon loss was especially high for C4-plants maize, Sorghum, and Sudan grass, whereas it was especially low for the legume pea.

The modelled parallel first-order decomposition kinetic, which separated two different carbon pools with different decomposition rates, concordantly fitted with the observed decomposition. Therefore,

predictions of C_{pot} for infinitive incubation duration provided precise estimations of the carbon fraction remaining in soil after decomposition and potentially contributing to SOC formation. The C_{pot} significantly differed between different plant residue types and crop species. For all crop species, C_{pot} of fine roots was higher than of straw, stubbles, litter, and coarse roots (Table 15). High stability of fine roots in decomposition have been shown before (Wilhelm et al., 2004, Rasse et al., 2005) and ascribed to as chemical recalcitrance of root tissues, physicochemical protection of roots from microbial access, which evolve through the intensive contact between roots and soil particles. For all 6 crop species in all cropping systems, except for pea and winter cereal, the C_{pot} of litter was slightly higher than C_{pot} of stubbles and coarse roots. In contrast to other residue types, the C_{pot} of litter was minimally influenced by the crop species and varied within a narrow range from 210 g (kg EOC)$^{-1}$ for winter cereal and pea to 300 g (kg EOC)$^{-1}$ for maize (Table 15). Litter consisted of senescent leaves which were collected in several samplings from the soil surface. Irrespective of crop species, the leave tissues of litter were chlorotic and mainly necrotic. It was expectable that large portions of the soluble carbon fraction had already been translocated into the green shoot, leached via pouring rain, and used by instantaneous microbial access, which all covered an influence of crop species on the decomposability of litter.

The C_{pot} of C4-plants depended on the position in the cropping system. For maize, C_{pot} of litter was higher for the main crop than for the second crop, whereas C_{pot} of fine roots was higher for the second crop than for the main crop. The C_{pot} of coarse roots for winter cereal depended on the phenological stage. An influence of the phenological stage on mineralisation of plant residues was reported by Bertrand et al. (2009). The interaction of phenological stage and plant residue type on decomposability might be due to different involvement of vegetative plant parts into reallocation of non-structural organic carbon compounds. The translocation of non-structural carbon compounds from leaves, stems and coarse roots to the generative plant organs might have been less completed in an early phenological stage. Litter tissues therefore were more chlorotic than necrotic, stubbles and coarse roots contained more easily decomposable carbon compounds, resulting in less C_{pot} of all crop residues except fine roots.

The C_{pot} for crop residues of the intercropping systems maize-Sorghum and pea-oats differed from the arithmetic mean of both C_{pot}-values for the involved crop species in single cropping. The intercropping residues often similarly decomposed to the residues of one of both species (Table 15). The proportions of each species at the intercropping crop yield (Höcker et al., 2015) could not explain this phenomenon in every case: In case of pea-oats, the C_{pot} of the litter and stubbles was similar to those of oats, which equally dominated the crop yield. However, the C_{pot} of coarse roots was much lower for pea-oats than the arithmetic mean of C_{pot} for pea and oats in single cropping. In case of maize-Sorghum, the C_{pot} of litter and stubbles was similar to those of maize, while Sorghum dominated the crop yield. Coarse roots of maize-Sorghum even reached a higher C_{pot} than coarse roots of maize or Sorghum in single cropping. Such non-additive patterns of plant residue mixtures have already been reported (Gartner and Cardon, 2004). As there is no evidence for additive effects of litter mixing on decomposition by intercropping

(Cong et al., 2015), singly biochemical alterations due to interspecies competition, which have not been detected by the applied biochemical properties could explain this enhanced stability of intercropping residues.

3.4.3 Is C_{pot} of plant residues predictable by biochemical properties?

As persistence of soil organic matter is an ecosystem property, insignificant of molecular structure (Schmidt et al., 2011), persistence of EOC, such as plant residues, was determined in incubation experiments (incubation) experiments, instead of deducing from long-term field experiments. The decomposition of plant residues from energy-crop cultivation was measured in two incubation experiments, which both contained wheat straw as standard material. The decomposition of this standard residue slightly deviated in both experiments, especially at the end of incubation (Figure 22). In the second experiment, which predominantly contained fine roots as plant residue, the total carbon loss of the standard material was about 10 per cent lower than in the first incubation experiment. Both incubation experiments were conducted one after another over a period of 2 years, wherefore a separate soil collect for each incubation became necessary. Despite both soils were collected at the same site, the second one contained more mineral nitrogen in the soil solution, which enhances nitrogen availability for microorganisms and therewith increases the portion of the standard residue remaining and contributing to soil organic matter (Kirkby et al., 2013, 2014). This portion is expressed as C_{pot} and measured as portion of organic carbon, which is applied to soil as plant residue and is not mineralised (the total carbon loss) in the incubation experiment, and is assumed to be decomposed in the long-term under out-door conditions at the same rate as soil organic matter (Lashermes et al., 2009). This way, C_{pot} characterises the reproduction of SOC by a certain plant residue and is a measure of persistence of this plant residue in soil. In order to compare C_{pot} of all observed plant residues and to correlate biochemical quality and decomposition, a correction factor was calibrated at the standard residue in order to stretch every measured decomposition kinetic in the second experiment. Assuming the different N availability had influenced decomposition of other plant residues likewise, decomposition in both incubation experiments was made comparable with this correction. The correlation analysis was conducted to prove the significance of the relation between biochemical parameters and C_{pot} for a future prediction of C_{pot} by biochemical properties.

In contrast to previous investigation (Jensen et al., 2005, Abiven et al., 2005, Lashermes et al., 2009), both incubation experiments were conducted without mineral N supply, as there is still a lack of evidence for decomposition to appear unlimited from mineral N-availability in cultivated fields. A limitation of decomposition was expected, especially for plant residues with a high initial C/N-ratio like stubbles and coarse roots in the intermediate decomposition stage, between 3rd and 56th day of incubation. In this period of incubation, both the lignin content and the lignin/N-ratio were negatively correlated with the course of EOC-induced CO_2-release. As lignin contains several phenolic carbon compounds, which are difficult to be accessed by microorganism, the availability of lignin-N for microorganisms requires

mineralisation at additional cost of energy (microbial N-mining), leading to reduced CUE (Craine et al., 2007). N-scarcity therefore not only limited decomposition of plant residues, but also decreased C_{pot} by a less efficient microbial carbon use, especially of those plant residues with low available N in plant tissues (high C/N-ratio, high lignin/N-ratio). Both C/N-ratio and lignin/N-ratio were negatively correlated with C_{pot}, whereas the lignin concentration was positively correlated with C_{pot}.

The C_{pot} was negatively correlated with the cellulose concentration, equally. However, the influence of cellulose on decomposition has been contentiously characterised in incubation experiments, which prevented any limitation of nitrogen availability. A negative influence of cellulose on total carbon loss has been identified for plant residues (Jensen et al., 2005), while no influence appeared for a broad range of EOC (Lashermes et al., 2009). Cellulose is a typically structural component of plant cell walls and of a high carbon concentration. Nitrogen scarcity therefore might have essentially reduced the efficiency, at which microorganisms used cellulose (Manzoni et al., 2010).

The conventional understanding, that plant residues persist in soils according to the 'recalcitrance' of their chemical compounds (Marschner et al., 2008), which describes the resistance of a chemical compound towards microbial decomposition has been recently updated by the framework of microbial carbon use efficiency (Cotrufo et al., 2013). This framework emphasizes the formation of stable decomposer products from predominantly easily available carbon compounds such as water-soluble carbohydrates as dissolved-organic-matter-pathway of SOC formation (Cotrufo et al., 2015). However, water-soluble carbohydrates are also suspected to cause a substantial mineralisation of already stabilised organic matter (positive priming effect) in soil (Paterson and Sim, 2013, Mazzilli et al., 2014). In this investigation, a significantly negative relation between C_{pot} and the concentration of water-soluble carbohydrates occurred. The detection of C_{pot} rather describes an effect of plant residue application on soil than the carbon fraction of plant residues, which was converted into SOC, as EOC-induced CO_2-release is not assigned to the mineralised carbon source by isotopic tracing. The negative relation between C_{pot} and water-soluble carbohydrates supported a positive priming effect, but equally did not exclude a substantial formation of SOC from water-soluble carbohydrates, which was counterbalanced by the priming effect. As the concentration of water-soluble carbohydrates was tightly correlated with the EOC-induced CO_2-release in the initial 3 days of incubation, these processes occurred in the beginning of the experiment. The concentration of water-soluble carbohydrates constituted an alternative to the C_{3d}-value in the current biochemical indicator by Lashermes et al. (2009).

Lashermes et al. (2009) developped an indicator of C_{pot} (I_{pot}) for a variety of EOC by a partial least squares regression of biochemical properties and C_{pot}, which was determined in incubation experiments. Thereby either the fibre fractions SOL, HEM, CEL, and LIC were exclusively used as predictive parameters or together with the C_{3d}-value, which expressed the EOC-induced CO_2-release in the initial 3 days of incubation. The validation of both indicator proposals for the appropriate data of 40 plant residues in this investigation showed highly significant relations, whereby the coefficient of

determination was higher, if not only biochemical parameters but also the C_{3d}-value was included into the predictive variables (Table 17). However, it was obvious, that the regression line of experimentally determined and predicted C_{pot}-values largely deviated from the 1:1-line and also failed the point of origin (Figure 25).

In the current indicator by Lashermes et al. (2009), data from several incubation experiments was used, which were conducted under differently controlled environmental conditions (e.g. soil, incubation duration, C addition / incubation ratio). Furthermore, this set of data solely partially contained plant residues (14 plant residues of 83 EOC samples). Therefore, this investigation aimed to set up an indicator for exclusively plant residues by (i) a simple recalibration of the current indicator, and (ii) a reconstitution by the integration WSC and the total N concentration in plant residues. Both steps increased the coefficient of determination of either the indicator type based on biochemical parameters or the one based on initial EOC-induced CO_2-release and biochemical parameters. The difference between the coefficients of determination of both indicator types thereby largely diminished, and under consideration of the experimental effort of incubation experiments to assess the C_{3d}-value, the regression type A, which is exclusively based on biochemical parameters can be preferred for an indicator.

For the reconstituted indicators, the relation between measured and predicted C_{pot}-values virtually accorded, but the majority of the ordered pairs of numbers for fine roots were still located below this regression line, whereas the ordered pairs of coarse roots were located above the regression line (Figure 25). This implies, that the biochemically indicated values systematically underestimate C_{pot} of fine roots, whereas they overestimate C_{pot} of coarse roots. Thus, the higher coefficients of determination and the course of the regression line between predicted and measured C_{pot}-values show, than the reconstituted indicator is more appropriate to predict C_{pot} of plant residues than the current indicator by Lashermes et al. (2009).

3.5 Conclusion

The objective of this investigation was to compare biochemical quality and course of EOC-induced CO_2-release of plant residues in energy-crop cultivation to evaluate the effect of maize, sorghum, and Sudan grass (C4-plants) cultivation on soil organic matter towards the background of C3-plants. The course of EOC-induced CO_2-release of plant residues was specific for crops and cropping systems. C4-plants contained substantially more water-soluble carbohydrates in stubble and coarse root, revealing higher decomposability of the crop residue but less C_{pot} than winter cereal and pea. Solely litter appeared to be unspecific in biochemical quality and course of EOC-induced CO_2-release for crops and cropping systems. Fine roots revealed the highest C_{pot}, requiring further insights into accessibility. In double cropping, C_{pot} of above-ground residues was decreased, but C_{pot} of fine roots was increased. Maize-sorghum intercropping affected the decomposability of plant residues beyond simple additive effects. The measured course of EOC-induced CO_2-release could be modelled with the parallel first-order decomposition kinetic, allowing the determination of C_{pot}. This experimentally determined fraction of

plant residues, which remains in soil and contributes to SOC, was closely correlated to biochemical properties and was therefore predictable. The current indicator I_{pot} for EOC samples by Lashermes et al. (2009) could be reconstituted to an indicator, which specifically predicts C_{pot} for plant residues, based on nitrogen (N), water-soluble carbohydrates (WSC), cellulose (CEL), lignin (LIC):

$$I_{pot} = 269 + 13\,N - 0.5\,WSC + 0.7\,CEL + 1.5\,LIC.$$

4 Does biochemical quality of exogenous organic carbon predict potential residual organic carbon in soil?

4.1 Introduction

Strategies for restoration of soil organic carbon stocks (SOCS) not only increase fertility, it might contribute to mitigation of greenhouse gas accumulation by sequestering organic C into soil (Rice, 2006). In cultivated soils, the restoration of SOCS depends on the incorporation of plant residues into soil and the application of EOC, which do not origin from agricultural primary production. Thereby, the proportion of exogenous organic C (EOC) remaining and contributing to organic C accumulation in soil, results from its recalcitrance to decomposition or from incorporation into soil organic pools. The quantification of this portion of EOC is of substantial interest for soil organic carbon (SOC) balancing (Andren and Katterer, 1997, Bolinder et al., 2008) and for the evaluation of crop species, organic amendments, and cropping systems in the agronomical approach on sustaining soil fertility (Brock et al., 2013, Ebertseder et al., 2014). This portion of EOC could be either quantified with use of litterbags in field experiments (e.g., Kou et al., 2015) or controlled environmental conditions (e.g., Birouste et al., 2012) or alternatively in incubation studies under controlled environmental conditions (e.g., Redin et al., 2014). Classically, soil laboratory incubation studies are used to assess decomposition of EOC over different periods of time. Although initial effects of EOC application on microbial soil respiration could be excellently observed in laboratory incubations (Thuries et al., 2002, Morvan et al., 2006), the residual portion of EOC, which contributes to organic C accumulation in soil requires a mathematical determination (Lashermes et al., 2009). Irrespective of incubation duration, experiments under controlled environmental conditions solely reproduce the initial decomposition process (herein referred to as ‘decomposition’), as long-term decomposition rather depends on ecosystem properties than on EOC application (Schmidt et al., 2011).

The C and N mineralisation during incubation basically depends on biochemical quality of EOC, expressed as concentration of C and N in EOC (Jensen, 1929, Nicolardot et al., 2001), the proportion of C and N in soluble organic matter (SOL), hemicellulose (HEM), cellulose (CEL), and lignin (LIC) as determined by proximate analysis (also referred to as ‘stepwise chemical digestion’) (Henriksen and Breland, 1999, Trinsoutrot et al., 2000, Thuries et al., 2002, Ruffo and Bollero, 2003, Jensen et al., 2005, Morvan et al., 2006), near-infrared spectroscopy (Bruun et al., 2005, Henriksen et al., 2007, Borgen et al., 2011, Peltre et al., 2014), or NMR-spectroscopy (Baldock et al., 1997, Wang et al., 2004, Mathers et al., 2007, Bonanomi et al., 2013).

The relation between decomposition and biochemical quality of EOC has motivated investigation on biochemical indication of the proportion of EOC remaining and contributing to organic C accumulation in soil. Although biochemical indicators based on (i) the concentration of C and N in EOC (Robin, 1997), (ii) the proportion of C in biochemical fractions (Thuries et al., 2002, Lashermes et al., 2009), (iii) the proportion of organic C mineralized during 3 days of incubation and the proportion of C in

biochemical fractions (Lashermes et al., 2009) have been proposed, the agronomical approach uses long-term field experiments to evaluate the proportion of EOC remaining and contributing to organic C accumulation in soil (Brock et al., 2013). The indicator of I_{pot} has been developed for the main types of EOC, encompassing plant residues, mulches, organic fertilisers, composted manures, urban composts, and wastewater sludge (Lashermes et al., 2009) and requires a validation for recently emerging EOC.

The production of biogas provides a separate EOC group: Digestates (word composition of 'digested substrate'), residues of microbial fermentation of numerously combined agricultural, industrial, and urban substrates. Fermentation involves CO_2 release, thereby decreases the magnitude of returned carbon to soil (Thomsen et al., 2013) but also changes biochemical quality of returned carbon to soil. In digestates, the C concentrations are decreased, while N concentrations are increased, compared to the original substrates. Microbial fermentation likewise decreases the proportion of C in low-molecular carbon compounds, whereas the proportion of C in lignin is increased. Due to these changes in biochemical quality, the application of digestates instead of the original substrates diminishes microbial activity in soil (Ernst et al., 2008) and even may diminish levels of organic matter in soil (Verloop et al., 2015). The biochemical quality and the course of EOC-induced CO_2-release of digestates largely varies, depending on the original substrates (Alburquerque et al., 2012), fermentation temperature (Lu et al., 2014), a solid phase separation (Grigatti et al., 2011), and post-composting of digestates (Bustamante et al., 2012, Torres-Climent et al., 2015). However, the characterisation of the residual portion of EOC, which contributes to organic C accumulation in soil largely varies for digestates from negative values due to excessive positive priming effects of fermented sludge (Bernal and Kirchmann, 1992), to positive values comparably to farmyard-manure (Moorhead et al., 1987, Sanger et al., 2011), to even complete stability of digestates in soil (Kolar et al., 2008).

The application of pyrogenic organic matter in various types of biochar amendments in European soils can be retraced until the 19th century and recently emerges (Abiven et al., 2014). Pyrogenic organic matter is thermally transformed organic material under anoxic conditions (pyrolysis). Pyrolysis can occur during wildfires, where local and temporary limitation of oxygen can occur, or it can be a controlled process to produce heat and pyrogenic organic matter (PyOM) from agricultural residues (Maestrini et al., 2015). In the presence of high pressure and water, pyrolytic processes occur at lower temperature, referred to as hydrothermal carbonisation (HTC) of biomass residuals and digestates (Libra et al., 2011, Mumme et al., 2011). The thermal transformation of organic matter encompasses dehydration and depolymerisation of plant biopolymers to volatile dissociation products (i.e. sugars, pyrenes, and furans), while aromatic structures of lignin accumulate and condense with aliphatic intermediates (i.e. quinones) to porous crystallites (Keiluweit et al., 2010). As condensation and crystallisation strongly depend on the pyrolysis temperature (Keiluweit et al., 2010), hydrothermally carbonised chars contain more volatile compounds (Malghani et al., 2013), are less persistent in soil, and cause more SOC priming than PyOM in the classical meaning (Bamminger et al., 2014). Pyrogenic

organic matter (also referred to as 'black C', biochar, and fire residues) persists for centuries (Hammes et al., 2008) and was suspected to persist longer than bulk organic matter in soil (Schmidt and Noack, 2000, Schmidt et al., 2011). Therefore, the production of biochar has been proposed as methodology to substantially enhance carbon sequestration in soil (Lackner, 2003, Lehmann, 2007, Abiven et al., 2014).

The biochemical properties, which mainly influence decomposition specifically differ between crop residues, digestates, and biochar. The proportions of C in low-molecular carbon compounds, cellulose, and lignin control decomposition of plant residues, whereas digestates contain a large proportion of microbial biomass carbon, underlying slow decomposition (Coban et al., 2015). There is a lack of knowledge how different biochemical fractions, e.g. lignin, hemicellulose, and cellulose might be used to estimate the proportion of EOC remaining and contributing to organic C accumulation in soil for different types of EOC, which originate from microbial or thermal conversion of plant-derived C.

We collected samples of straw, digestates, urban composts, and biochar from different source areas, spread over Germany and Switzerland. For assessment of decomposition we measured for 310 days the EOC-induced CO_2-release from soil in incubation experiments under controlled environmental conditions. The course of EOC-induced CO_2-release was evaluated with a model which divides the carbon into potentially biodegradable and residual pools, and allows the determination of the mean transit time of the biodegradable carbon pool in soil (Manzoni et al., 2012a). Our main hypothesis was that parameters of biochemical quality are insensitive for biochemical alterations due to thermal or microbial conversion, and therefore do not predict C_{pot} by a common indicator for all groups of EOC.

4.2 Material and Methods

4.2.1 EOC

A collection of 30 exogenous organic carbon sources (EOC) was constituted, ranging from common materials in agriculture and urban gardening, like straw, farmyard manure and composts derived from different origins and compositions. Additionally biochars and digestates from biogas production, as well as different plant residues from Sorghum (*Sorghum bicolor* L. Moench) were investigated (Table 19). Of these, 26 % were plant residues, 63 % were manures, and 10 % char. Plant residues from Sorghum and Wheat originated from a field experiment in Berlin Dahlem, cultivation year 2012 (Höcker et al., 2015). Manure, containing materials of different microbial processing, was further grouped into urban composts (30 %), farm fertilisers (13 %), and digestates (20 %). Char contained beneath biochar hydrothermal carbonised materials, whereof wheat shoot was pyrolysed at 200 °C for 3 h and poplar wood was pyrolysed at 200 °C for 20 min. The source area of the EOC samples spread over Germany and Switzerland. During the sampling process, the initial consistence and moisture content were kept. Depending on the initial consistence, the EOC samples were cut into pieces of 1 mm particle size by milling (RETSCH® SM 2000), free cutting, or disperging (IKA® ULTRA-TURRAX) for dry, moist, and liquid EOC samples, respectively.

A subsample of each EOC sample was taken and finely ground in a Retsch® ball mill. The pulverised subsample was analysed for total carbon (EN 15936) and total nitrogen concentrations (EN 16168), using elementary analysis (elementar® varioMAX®) after dry combustion (Dumas, 1831). The fibre fractions hemicellulose (HEM), cellulose (CEL) and lignin (LIC) were determined for each EOC sample by their solubility in detergents (neutral detergent solution, acid detergent solution, and a concentrated sulfuric acid solution) as described for determination of plant cell wall constituents (Van Soest and Wine, 1967) and used for determination of forage digestibility (following the German standard VDLUFA, 1976). All fibre fraction determinations were conducted in a half-automatic digestion apparatus (FOSS® Fibertec™ 1020) as repeat determination with further laboratory repetitions, until three values deviated less than three per cent from their arithmetic mean (n = 1, N = 30). The HEM fraction was calculated as difference of neutral (NDF) and acid detergent fibre (ADF), the CEL fraction was calculated as difference between ADF and acid detergent lignin (ADL), and ADL was accounted as LIC. In addition to all fibre fractions, a soluble fraction (SOL) was calculated as difference between initial dry matter and NDF, containing both crude ash and the soluble organic matter in neutral detergent solution. Dry matter (DM) was determined at 105 °C. Furthermore, the concentration of water-soluble carbohydrates (WSC) was determined in a cold-water extract of 0.5 g EOC dry matter in 100 ml deionised water, using the anthron method (according to Yemm and Willis, 1954, adapted by von Lengerken and Zimmermann, 1991) and spectrometrically detected in continuous flow analysis (CFA). A repeat determination of WSC was conducted with the criterion of five per cent deviation from arithmetic mean. All element levels and biochemical fractions were expressed in g $(100\ g\ DM)^{-1}$ for characterisation of biochemical quality and in g $(kg\ DM)^{-1}$ for parameterisation of the indicator I_{pot}.

Table 19 Groups, types, source area, and applied consistence of 30 EOC samples.

Group	Type	Sample	Consistence	Source area, Institution
Plant residue				
Straw	Wheat straw	1	solid, dry	Berlin
		2	solid, dry	ZALF Müncheberg
	Rape straw	3	solid, dry	Berlin
	Rye straw	4	solid, dry	Berlin
Energy crop	Sorghum litter	5	solid, dry	Berlin
residue	Sorghum stubble	6	solid, dry	Berlin
	Sorghum coarse root	7	solid, dry	Berlin
	Sorghum fine root	8	solid, dry	Berlin
Manure				
Urban	Organic waste compost	9	solid, moist	Berlin
compost		10	solid, moist	Luebeck
	Municipal solid waste compost	11	solid, moist	Saxony
		12	solid, moist	Luebeck
	Organic waste & municipal solid	13	solid, moist	Regen
	waste compost	14	solid, moist	Soest
		15	solid, moist	Alzey-Worms
		16	solid, moist	Saxony
	Green waste compost	17	solid, moist	Berlin
Farm	Farmyard manure	18	solid, moist	ZALF Müncheberg
fertiliser	Farmyard manure (rotted)	19	solid, moist	Thyrow
		20	solid, moist	Groß-Kreutz
	Cattle slurry	21	liquid	Berlin
Digestate	Organic waste	22	liquid	Lemgo
	Organic waste (separated)	23	liquid	Berlin
	Maize silage	24	liquid	Berlin
	Maize silage & Slurry	25	liquid	Berlin
	Maize silage & Slurry	26	solid, moist	Saxony
	Maize silage & Slurry & Farmyard manure & Organic waste (cofermentation)	27	solid, moist	Berlin
Pyrogenic organic matter (PyOM)				
Char	Biochar	28	solid, dry	Switzerland, Ava-CO_2
	HTC-wheat straw	29	solid, dry	Leibniz Institute for Agricultural Engineering Potsdam
	HTC-poplar wood	30	solid, dry	

4.2.2 Incubation experiment

4.2.2.1 Soil and site

A sandy loam, containing 1.6 % organic carbon, 0.11 % total nitrogen was collected from a long-term field experiment in Berlin Dahlem with intensive farm yard manure application over the past decades. Soil sampling occurred at three points (replications) along a 20 m line in the field for a pooled sample, representing the upper 30 cm of the A-horizon. The soil was sieved without drying to 2 mm particle size and stored for 10 days at 22 °C.

4.2.2.2 Setup of the incubation study

The EOC were homogeneously mixed with soil at a rate of 400 mg EOC per 100 g soil. Then the soil was filled into small tubes (soil columns) at a bulk density of 1.1 g cm^{-3}. Soil columns with and without EOC were prepared with 4 replications (30 EOC samples × 4 replications + no EOC × 4 replications, N = 124). Mineral N was supplied as KNO_3 at a rate of 100 mg N (kg soil)$^{-1}$ to prevent the decomposition process from limited nitrogen availability (Henriksen and Breland, 1999). Additionally, mineral phosphorus was supplied as Mg_2PO_3 at a rate of 20 mg N (kg soil)$^{-1}$ to prevent the decomposition process from limited phosphorus availability (Kirkby et al., 2013). Incubation temperature was 22 °C. At the start of incubation, soil water content was adjusted to 50 % of water holding capacity (ISO 16072), by adding 19.1 ml H_2O per 100 g soil.

4.2.2.3 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.2 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.4 M HCl and phenolphthalein as indicator. Since 21st day of incubation, the concentration of measurement solutions was reduced to 0.1 M NaOH and 0.4 M HCl, while the amount of $BaCl_2$ addition for CO_2 precipitation was reduced to 7.5 ml. Measurement dates were 1, 3, 7, 14, 21, 35, 56, 77, 98, 120, 162, 217, and 310 days after start of incubation. The apparent decomposition of EOC was calculated as difference between evolved CO_2 from soil columns with and without EOC. The course of EOC-induced CO_2-release was calculated by summing up the EOC-induced CO_2-release between two subsequent measurement dates. The apparent decomposition of EOC in soil was expressed as EOC-induced CO_2-release in g C (g EOC)$^{-1}$. The EOC-induced CO_2-release is named "apparent" decomposition, as it integrates SOC priming and EOC mineralisation.

4.2.3 Statistical analysis and modelling

4.2.3.1 Statistical analysis of biochemical quality

Results are presented as means (least square means) of EOC types and samples of different source areas for each EOC type (5 EOC groups, 23 EOC types, 30 EOC samples, N = 30). To compare the different EOC groups, for each biochemical parameter a linear mixed model was set as hierarchically classified:

$$y_{ij} = \mu + a_i + b_{j(i)} + \varepsilon_{ij}$$

where a_i is the EOC group and $b_{j(i)}$ is the EOC type in each EOC group. Models were set up under consideration of the co-variance structure and Kenward-Roger degrees of freedom approximation. Overall means of EOC groups were calculated as unweighted least square means. The effect of the EOC group was tested with an F-test and consecutively distinguished by pairwise comparisons, using Tukey-HSD-test (α at the 0.05 probability level). All statistical analyses were conducted in SAS® 9.2.

4.2.3.2 Potential residual organic carbon

The course of EOC-induced CO_2-release was modelled by the D2 and D3 models (details in section 2.2.5.1 on page 31). Potential residual organic carbon (C_{pot}) was determined for each EOC sample (N = 30) as described in chapter 3.2.3.2 on page 67 and statistically evaluated equal to biochemical properties in section 4.2.3.1.

4.2.3.3 Indicator I_{pot}

At first, the elaborated indicator of C_{pot} (I_{pot}) in g C (kg EOC)$^{-1}$, which has been set up for the main types of EOC (Lashermes et al., 2009), was validated for the set of EOC samples containing the emerging EOC types digestate and char beneath plant residues, manures and urban composts (Validation). The validation criterion was the coefficient of determination R^2, calculated by the Pearson correlation coefficient r, and the adjusted determination coefficient R_a^2 which is the corrected R^2 by the number of regression variables.

Secondly, the indicator was recalibrated by partial least squares regression analysis (Recalibration). The dependent regression variable was C_{pot} in g C (kg EOC)$^{-1}$, predicted by the fibre fractions *CEL,* and *LIC* in g (kg DM)$^{-1}$ and the EOC-induced CO_2-release within the first 3 days of incubation C_{3d} in g C (kg EOC)$^{-1}$ as independent regression variables. The soluble fraction (*SOL*) and hemicelluloses were not respected as predictive variables, as negative values occurred for hemicelluloses even in previous investigation (Lashermes et al., 2009), disqualifying the both of them as predictive variables.

Finally, the indicator was reconstituted, integrating total carbon concentration *C* and water-soluble carbohydrates *WSC*, both in g (kg DM)$^{-1}$, into regression (Reconstitution). As previous studies (Lashermes et al., 2009) gave evidence for normal distribution of all independent variables, normality was assumed in this study and variables were not transformed. The multi-collinearity of the independent regression variables was revealed in previous investigations (Jensen et al., 2005, Lashermes et al., 2009) and occurred in this study as well. The partial least squares regression procedure overcomes this problem through analysing principal components (factors *F*) out of the magnitude of independent regression variables, and constituting the regression on them (Bruun et al., 2005, Lashermes et al., 2009). To select the optimal number of partial least squares components, the "leave-one-out-cross-validation" method was used. The partial least-squares regression was always run with a complete set of initial regression variables (either biochemical properties or biochemical properties and initial EOC-induced CO_2-release C_{3d}), successively leaving out variables, if they were declared to be unimportant by the Wold criterion *VIP* < 0.8 or if they reached regression coefficients < 0.2 in the centred and scaled solution.

4.3 Results

4.3.1 Biochemical quality of EOC

The biochemical quality was dependent on EOC group (plant residues, urban compost, farm fertiliser, digestates, and char) as each biochemical property value for a biochemical parameter (C/N-ratio, WSC, HEM, CEL, and LIC) differed between EOC groups (at the 0.001 probability level, F-test of the two-way-ANOVA as hierarchically classified by EOC group and EOC type) (Table 20). Biochemical quality largely varied between EOC types of plant residues (e.g. litter, stubble, coarse root, and fine root in the group of crop residues) and char, but remained uniform for different groups of manures (farm fertiliser, digestates, urban composts).

Characteristically, plant residues contained large proportions of hemicellulose and cellulose, which were highest of all EOC groups, but the least proportion of lignin. Plant residues reached relatively high C/N-ratios, especially for straw. Although crop residues of Sorghum were of lower C/N-ratio, they contained high proportions of water-soluble carbohydrates, especially in stubble, coarse root, and fine root, 255, 158, and 47 g (kg DM)$^{-1}$ respectively. Fine root contained 107 g (kg DM)$^{-1}$ lignin, similar to rape straw.

Manure represented a broad range of EOC, which had already passed microbial processing, either by composting to urban compost, by digestion to farm yard manure, or by fermentation to digestate. Manures were therefore grouped according to the type of microbial processing. Irrespective of microbial processing, all manures contained virtually no water-soluble carbohydrates. Concordantly to previous findings (Lashermes et al., 2009), some manures but all urban composts were characterised by apparently negative hemicellulose contents, issuing the applicability of the stepwise chemical digestion method (Van Soest and Wine, 1967) for these types. However, there were differences in the proportion of cellulose among these groups of manures. Cellulose contents differed between urban compost and farmyard manure, reaching on average 88 and 208 g (kg DM)$^{-1}$, respectively. In case of digestates, cellulose contents largely varied from 66 to 300 g (kg DM)$^{-1}$ depending on the original substrate of the fermentation. The proportion of lignin varied for different types of digestates between 142 and 243 g (kg DM)$^{-1}$ but even so for farm fertilisers and urban composts, whereas these groups of manures could not be distinguished in this biochemical property. Manures contained significantly more lignin than plant residues, but less than char. The C/N-ratio of manures was low, which contrasted both plant residues and char. Farm fertiliser were characterised by significantly higher C/N-ratio than urban compost, 17 and 13, respectively. Digestates, which were characterised by C/N 17 could not be distinguished from the both of them.

Pyrogenic organic matter (char) could be biochemically distinguished from plant residues and manures. Char was characterised by C/N 99, which was higher than for manures. Biochar, being dry carbonised was free of water-soluble carbohydrates, whereas hydrothermally carbonised wheat straw and poplar wood contained 0.8 and 33 g (kg DM)$^{-1}$. Furthermore, char contained 262, 557, and 604 g (kg DM)$^{-1}$ ‘lignin-like’, in HTC-poplar wood, HTC-wheat straw and biochar, respectively.

Table 20 Biochemical quality of types and groups of EOC: C/N-ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin. Means (least square means) of EOC samples (N = 30): Different lower case letters behind means (a-c) in columns indicate significant (t-Test, α = 0.05) differences among EOC groups.

Group	Type	Sample	CN	WSC	HEM	CEL	LIC
				g (kg DM)$^{-1}$			
Plant residue	Wheat straw	1	53	10.23	291	351	70
		2	133	5.02	305	483	87
	Rape straw	3	81	6.11	164	476	106
	Rye straw	4	121	7.34	324	446	75
	Sorghum litter	5	34	9.21	257	318	43
	Sorghum stubble	6	84	255.52	221	297	67
	Sorghum coarse root	7	70	158.59	197	220	66
	Sorghum fine root	8	44	46.95	307	213	107
	Mean (plant residue)		**77a**	**62a**	**258a**	**350a**	**78a**
Urban compost	Organic waste compost	9	13	2.15	-88	104	197
		10	13	0.66	-64	84	153
	Municipal solid waste compost	11	12	1.33	-119	79	164
		12	12	1.09	-68	82	176
	Organic waste & municipal solid waste compost	13	11	1.44	-29	98	158
		14	11	1.31	-61	103	159
		15	16	1.01	-43	107	163
		16	11	1.53	-85	69	126
	Green waste compost	17	18	0.80	-17	71	163
	Mean (urban compost)		**13b**	**1a**	**-64b**	**88b**	**162b**
Farm fertiliser	Farmyard manure	18	17	3.50	144	271	189
	Farmyard manure (rotted)	19	14	1.67	14	127	261
		20	19	2.88	81	273	218
	Cattle slurry	21	17	1.38	154	203	144
	Mean (farm fertiliser)		**17c**	**2a**	**98c**	**218c**	**203b**
Digestate	Organic waste	22	13	0.84	-17	66	142
	Organic waste (separated)	23	13	0.65	9	68	142
	Maize silage	24	16	1.23	-14	174	256
	Maize silage & Slurry	25	14	1.08	111	123	153
	Maize silage & Slurry	26	15	2.71	-26	80	143
	Cofermentation	27	28	0.54	144	300	243
	Mean (digestate)		**17bc**	**1a**	**34c**	**135bc**	**180b**
Char	Biochar	28	152	0.36	-60	20	604
	HTC-wheat straw	29	56	0.84	-24	158	557
	HTC-poplar wood	30	88	32.98	29	478	262
	Mean (char)		**99a**	**11a**	**-18bc**	**218bc**	**474c**

4.3.2 Decomposition and persistence of EOC in soil

4.3.2.1 The course of EOC-induced CO_2-release

Decomposition proceeded differently for groups, types (significant at the 0.001 probability level at all measurement dates, F-test of the two-way-ANOVA as hierarchically classified by EOC groups and EOC types), and samples of EOC (Figure 26). Plant residues released the most carbon in decomposition, especially straw (Figure 26, upper left image), which lost more than half of the added carbon amount within the initial 56 days of incubation and subsequently continued carbon release at lower rates. Crop residues were easily decomposable, especially stubble and coarse root could be largely decomposed, losing 800 mg C (kg EOC)$^{-1}$ in total. Litter decomposition abruptly converged towards a lower limit than stubble and coarse root after rapid initial decomposition, while fine roots lost least carbon of all crop residues in each of the proposed decomposition stages. Pyrogenic organic matter (char, Figure 26, upper left image), persisted in soil depending on original substrate and pyrolysis method. An evenly negative EOC-induced CO_2-release occurred for biochar since the 21st day of incubation, which apparently protected SOC from decomposition (negative SOC priming effect). The hydrothermally carbonised chars showed a positive carbon release, which was apparently lower than for the original substrate. The total carbon loss of winter wheat could be decreased by the hydrothermal processing from originally 800 to finally 100 mg C (kg EOC)$^{-1}$. Furthermore, HTC-wheat straw released even less CO_2 than HTC-poplar wood. Farm fertilisers (Figure 26, upper right image) were partially more stable than plant residues, but varied over a similar range. Cattle slurry was comparably labile and showed a similar course of EOC-induced CO_2-release to litter. Farmyard manure was more stable than cattle slurry, whereby the carbon release depended on the rotting degree. The course of EOC-induced CO_2-release from farmyard manures equalled the one of fine root in from 3rd day of incubation onward. Urban composts (Figure 26, lower left image) were the most stable EOC and showed similar courses of carbon release, losing in total less than 200 mg C (kg EOC)$^{-1}$. Municipal solid waste composts and green-waste composts were the most stabile among them, losing less than 100 mg C (kg EOC)$^{-1}$ in total. The mixed composts of municipal solid waste and organic waste showed different courses of EOC-induced CO_2-release, depending on the source area and biochemical quality. Digestates (Figure 26, lower right image) released less carbon than 400 mg C (kg EOC)$^{-1}$ in decomposition, but the course of EOC-induced CO_2-release varied depending on the substrate source. Digestates of maize silage with and without slurry were the most labile liquid digestates, whereby the one with slurry released more carbon than the one without slurry from 3rd to 56th day of incubation. The fermentation of organic waste provided a digestate, which lost more carbon in incubation experiments than the organic waste composts, providing evidence for an influence of the microbial processing type on persistence of EOC. Additionally, the extraction of the solid phase from digestates (also referred to as separation) influenced the course of EOC-induced CO_2-release in decomposition. The separated solid digestate of maize & slurry fermentation released 77 g C (kg EOC)$^{-1}$ in decomposition, which was significantly less than of the unseparated liquid one (compare the continuous and broken red lines), and even less than of the cofermented solid digestate.

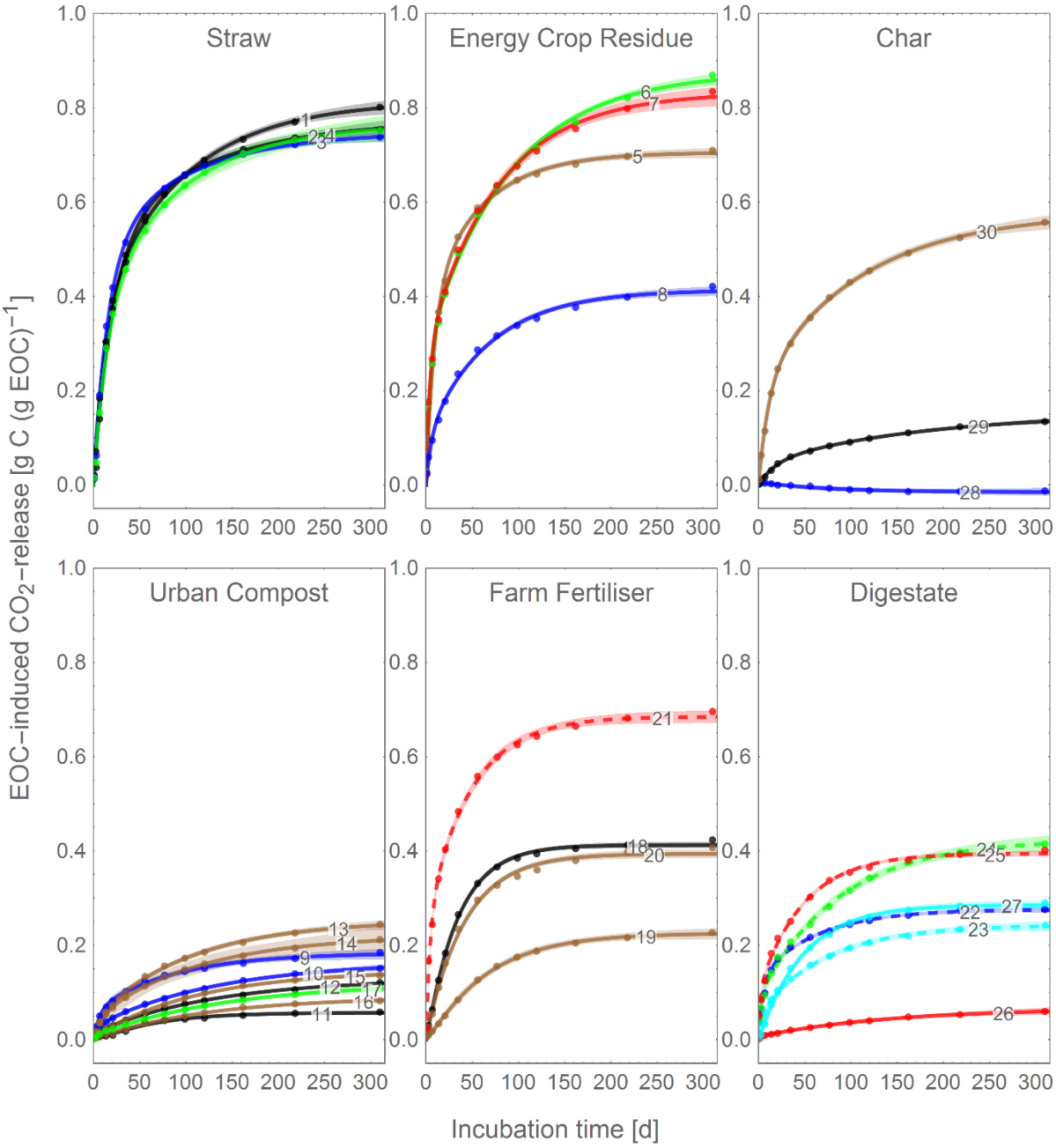

Figure 26 Decomposition of 30 EOC samples, categorised in EOC groups and EOC types. **Straw**: Winter wheat (black) [EOC sample 1, 2], Rape (blue) [3], Rye (green) [4]. **Energy crop residue**: Litter (brown) [5], Stubble (green) [6], Coarse root (red) [7], and Fine root (blue) [8] of sorghum. **Char**: Biochar (blue) [28], HTC wheat-straw (black) [29], HTC poplar-wood (brown) [30]. **Urban compost**: Organic waste (blue) [9, 10], Municipal solid waste (black) [11, 12], Organic waste and municipal solid waste (brown) [13, 14, 15, 16], green waste (green) [17]. **Farm fertiliser**: Farm-yard manure (black) [18], Farm-yard manure rotted (brown) [19, 20], Cattle slurry (red dashed) [21]. **Digestate**: Organic waste (blue dashed) [22], Organic waste digestate separation (cyan dashed) [23], Maize silage (green dashed) [24], Maize silage & Slurry (red dashed) [25], Maize silage & Slurry (red) [26], and Cofermentation (cyan) [27]. Continuous lines indicate solid EOC, broken (dashed) lines liquid EOC. EOC-induced CO_2-release given as mean dots (n = 4), and D2 model ± 95 % CI (shaded). EOC Exogenous organic carbon.

4.3.2.2 Potential residual organic carbon

The C_{pot} differed between groups (significant at the 0.001 probability level, F-test of the two-way-ANOVA, as hierarchically classified by EOC groups and EOC types), types and samples of EOC (Table 21). The lowest C_{pot}, on average 266 g C (kg EOC)$^{-1}$, were obtained for plant residues, although Sorghum fine roots left 586 g C (kg EOC)$^{-1}$ after decomposition in soil. Irrespective of crop species, the C_{pot} of straw was about 200 g C (kg EOC)$^{-1}$. Sorghum litter left significantly more C_{pot} than straw, in detail 295 g C (kg EOC)$^{-1}$, whereas stubble left less C_{pot} than straw 124 g C (kg EOC)$^{-1}$ in soil. The C_{pot} of stubbles and coarse root of sorghum could not be significantly distinguished by the CI of the estimations.

The variability of C_{pot} was especially high for samples and types of certain groups of manure. Urban composts were commonly high persistent in soil, leaving on average 840 g C (kg EOC)$^{-1}$ in soil after decomposition, while farm fertiliser and digestates were less persistent, leaving on average C_{pot} 530 g C (kg EOC)$^{-1}$ and 718 g C (kg EOC)$^{-1}$, respectively. However, there were large differences between C_{pot} of different farm fertilisers. Farmyard manures left 587, 606, and 774 g C (kg EOC)$^{-1}$ in soil, whereby the magnitude of C_{pot} increased with the degree of decomposition (fresh or rotted). Cattle slurry, a liquid farm fertiliser, was less persistent than farmyard manure, leaving 315 g C (kg EOC)$^{-1}$ in soil. Digestates left more C_{pot} in soil than cattle slurry and partially even more C_{pot} than the fresh variant of farmyard manure. The C_{pot} varied depending on the type of substrate and the separation of the solid phase after fermentation. The lowest C_{pot} was obtained for a digestate of maize silage, leaving 576 g C (kg EOC)$^{-1}$ in soil. Slightly more C_{pot} left the digestate of maize silage and cattle slurry (601 g C (kg EOC)$^{-1}$), whereas the separation of the solid phase increased C_{pot} for this substrate type to 933 g C (kg EOC)$^{-1}$. Digestates of organic wastes left comparably high amounts of C_{pot} in soil, whereby the separation slightly increased C_{pot} from 724 to 757 g C (kg EOC)$^{-1}$. The separation digestate of co-fermented maize silage, organic wastes, cattle slurry, and farmyard manure left 714 g C (kg EOC)$^{-1}$, which was intermediate.

Pyrogenic organic matter (char) largely varied in C_{pot}-values, revealing a heterogeneous group of altering persistence in soil, i.e. less carbon of hydrothermal char was left in soil after decomposition than once applied, whereas biochar apparently left more carbon in soil than once applied, indicating a negative priming effect on soil organic matter (Maestrini et al., 2015). Biochar remained the most persistent EOC, leaving 1015 g C (kg EOC)$^{-1}$ after decomposition in soil. The C_{pot} of hydrothermal char differed between both carbonised substrates, as for HTC-wheat straw 846 g C (kg EOC)$^{-1}$ and for HTC-poplar wood 422 g C (kg EOC)$^{-1}$ were obtained. These C_{pot}-values of char were higher, except for fine roots of Sorghum, than the ones of plant residues, but did not differ from the ones of different groups of the microbial stabilised manures.

Table 21 C_{pot} in groups and types of 30 samples of EOC: Calculation with D2 model as mean ± 95 % CI (n = 4), given as mean if more than 1 subsample existed. Different lower case letters behind means (a-c) indicate significant (t-Test, α = 0.05) differences among EOC groups.

Group	Type	Sample	C_{pot} [g C (kg EOC)$^{-1}$]		
Plant residue	Wheat straw	1	185	±	23.8
		2	226	±	39.9
	Rape straw	3	252	±	18.9
	Rye straw	4	234	±	47.1
	Sorghum litter	5	295	±	12.0
	Sorghum stubble	6	124	±	18.5
	Sorghum coarse root	7	168	±	25.7
	Sorghum fine root	8	586	±	11.7
	Mean (plant residue)		**266**	**a**	
Urban compost	Organic waste compost	9	816	±	15.9
		10	834	±	16.5
	Municipal solid waste compost	11	943	±	5.4
		12	870	±	8.4
	Organic waste & municipal solid waste compost	13	747	±	18.9
		14	779	±	56.2
		15	853	±	9.4
		16	910	±	9.0
	Green waste compost	17	879	±	19.3
	Mean (urban compost)		**840**	**b**	
Farm fertiliser	Farmyard manure	18	587	±	6.7
	Farmyard manure (rotted)	19	774	±	14.0
		20	606	±	9.2
	Cattle slurry	21	315	±	13.7
	Mean (farm fertiliser)		**530**	**c**	
Digestate	Organic waste	22	724	±	6.8
	Organic waste (separated)	23	757	±	12.1
	Maize silage	24	576	±	22.4
	Maize silage & Slurry	25	605	±	6.1
	Maize silage & Slurry (solid)	26	933	±	19.5
	Cofermentation (solid)	27	714	±	9.6
	Mean (digestate)		**718**	**c**	
Char	Biochar	28	1015	±	9.8
	HTC-wheat straw	29	846	±	28.9
	HTC-poplar wood	30	422	±	30.3
	Mean (char)		**761**	**bc**	

4.3.2.3 Influence of biochemical quality on the course of EOC-induced CO_2-release and C_{pot}

Biochemical quality was closely correlated to the course of EOC-induced CO_2-release (Table 22). In the first three days of incubation, solely water-soluble carbohydrates and hemicelluloses were significantly positive correlated to EOC-induced CO_2-release, both indicating easily accessible carbon fractions for microorganisms. Inversely, lignin has been negatively related to EOC-induced CO_2-release since the 3rd day of incubation, indicating a minor easily available carbon fraction for microorganisms. So far, this initial correlation pattern concordantly confirmed previous investigation, but could not provide further evidence for C/N-ratio, total C-concentration, and cellulose to be negatively correlated to EOC-induced CO_2-release (Lashermes et al., 2009). In the intermediate decomposition stage, lasting until 56th day of incubation and accounting for substantial EOC mineralisation, a characteristic correlation pattern of EOC-induced CO_2-release to biochemical properties emerged. On the contrary to previous findings, in this investigation a positive relation of EOC-induced CO_2-release to the C/N-ratio, total C-concentration, and cellulose appeared. Therefore, the total carbon loss after 310 days of incubation was positively correlated to the concentrations of carbon, water-soluble carbohydrates, hemicelluloses, and cellulose in dry matter of EOC samples whereas it was negatively correlated to the lignin concentration.

Inversely, the correlation of total C concentration and C/N-ratio to the C_{pot} was significantly negative, whereby the magnitude of the correlation was higher for total C concentration in dry matter than for C/N-ratio. Although there was no significant correlation of the total N concentration in dry matter of EOC samples to the course of EOC-induced CO_2-release in incubation, a significantly positive correlation ($r = 0.37$) to the estimation of C_{pot} occurred. Water-soluble carbohydrates, hemicelluloses, and celluloses were negatively correlated to C_{pot}, whereby for hemicelluloses and celluloses the highest magnitude of the correlation coefficient occurred, 0.86 and 0.82, respectively. Lignin was positively correlated to ($r = 0.52$).

The course of EOC-induced CO_2-release was tightly correlated with itself. For example, the cumulative carbon release after 3 days of incubation already explained more than 50 % of the total variation in cumulative carbon release after 310 days of incubation ($r = 0.79$).

Table 22 Pearson correlation coefficients of EOC-induced CO_2-release C (t_i) at different incubation stages and initial biochemical quality of EOC (n = 30). C total carbon concentration, N total nitrogen concentration, C/N-ratio, WSC water-soluble carbohydrates, HEM hemicellulose, CEL cellulose, LIC lignin, C_{pot} Potential residual organic carbon. Correlations in bold denote $r^2 > 0.5$; (*) significant at $p < 0.05$; (**) significant at $p < 0.01$; (***) significant at $p < 0.001$.

	Pearson correlation coefficient (r)									
EOC decomposition				Biochemical quality indices				Stepwise chemical digestion		
	C_{3d}	C_{56d}	C_{120d}	C	C/N	N	WSC	HEM	CEL	LIC
Initial decomposition stage of the potentially biodegradable carbon pool C_s										
C_{1d}				0,28	-0,01	0,23	0,23	0,46*	0,23	-0,32
C_{3d}	**1**			0,30	0,20	-0,15	0,69***	0,57**	0,36	-0,40*
Intermediate decomposition stage of the potentially biodegradable carbon pool C_s										
C_{7d}	**0,92*** **			0,40*	0,36	-0,31	0,57**	**0,75*** **	0,61***	-0,50**
C_{14d}	**0,84*** **			0,48**	0,44*	-0,35	0,47**	**0,82*** **	**0,74*** **	-0,51**
C_{21d}	**0,81*** **			0,51**	0,46*	-0,35	0,44*	**0,84*** **	**0,78*** **	-0,50**
C_{35d}	**0,79*** **			0,52**	0,45*	-0,34	0,43*	**0,86*** **	**0,80*** **	-0,51**
C_{56d}	**0,78*** **	**1**		0,52**	0,45*	-0,33	0,44*	**0,87*** **	**0,81*** **	-0,51**
Final decomposition stage of the potentially biodegradable carbon pool C_s										
C_{77d}	**0,78*** **	**1,00*** **		0,52**	0,45*	-0,33	0,44*	**0,87*** **	**0,81*** **	-0,51**
C_{120d}	**0,78*** **	**1,00*** **	**1**	0,52**	0,45*	-0,33	0,46*	**0,87*** **	**0,81*** **	-0,51**
C_{310d}	**0,79*** **	**0,99*** **	**1,00*** **	0,51**	0,45*	-0,35	0,51**	**0,86*** **	**0,81*** **	-0,52**
Long-term biodegradable carbon pool *1-*C_s										
C_{pot}	**-0,78*** **	**-0,99*** **	**-0,99*** **	-0,51**	-0,46**	0,37*	-0,51**	**-0,86*** **	**-0,82*** **	0,52**

4.3.2.4 Biochemical indication of C_{pot} (Indicator I_{pot})

The C_{pot} was related to biochemical properties and to the EOC-induced CO_2-release in the first three days of incubation (Table 22). Thereof several regression models have been proposed to predict (or indicate) C_{pot} by these sets of parameters. The current indicator of C_{pot} (I_{pot}), proposed by Lashermes et al. (2009), is based on both relations (regression type B) and was validated to determine 95 % of the total variation in C_{pot} of all EOC samples ($R^2 = 0.95$) (Table 23, Figure 27). However, the proposal for an indicator I_{pot}, which is singly based on biochemical properties (regression type A in Table 23) was validated to account for 53 % of the total variation in C_{pot} of all observed samples ($R^2 = 0.53$), leaving 47 % of variation unexplained. Recalibrations and reconstitutions of the indicator I_{pot} were therefore motivated to improve this proposal. Firstly, both proposals of the indicator I_{pot} were recalibrated to the underlying set of 30 EOC samples. For recalibration, the EOC-induced CO_2-release in the initial 3 days of incubation (C_{3d}) and the fibre fractions were used as regression variables (SOL, HEM, CEL, and LIC), but on the contrary to previous investigation (Lashermes et al., 2009), hemicellulose (HEM) and the fraction of neutral detergent soluble organic matter (SOL) were not considered for the recalibration of an indicator, as unreliably negative values for HEM occurred in the stepwise chemical digestion. The partial least squares regression (PLS) worked in two steps, as it first analysed the main components, which were referred to as factors (F), out of the magnitude of provided independent regression variables, and in a second step correlated the analysed factors F to the dependent variable C_{pot}. The recalibration provided a slightly improved proposal ($R^2 = 0.75$) for regression type A. Despite the increased coefficient of determination, the graphical visualisation of calculated and predicted values (Figure 27, middle left graph) still revealed a lack of accuracy for low and high C_{pot}-values. This lack of accuracy motivated for a reconstitution of the independent regression variables by the integration of the total carbon concentration (C). This made cellulose (CEL) obsolete as regression variable and further improved the prediction of regression type A ($R^2 = 0.92$), especially for high C_{pot}-values (Figure 27, lower left graph).

The current indicator I_{pot}, which includes EOC-induced CO_2-release in the first 3 days of incubation (C_{3d}) into the regression variables (regression type B, Table 23) could neither be improved by the recalibration nor by the reconstitution, both keeping the current determination level of $R^2 = 0.95$. The comparison of calculated C_{pot}-values by the D2 model and appropriate estimations by the current indicator I_{pot} showed a good fit for both, high and low C_{pot}-values (Figure 27, upper right graph). This determination level was even higher than the one of the reconstituted indicator proposal, which was singly based on biochemical parameters (regression type A, Table 23). Although the regression type A could be largely enhanced by a reconstitution and recalibration, the current indicator I_{pot}, which follows regression B, still provided the best goodness of fit.

Table 23 Validation, recalibration, and reconstitution of current partial least squares (PLS) regressions predicting potential residual organic carbon C_{pot} in EOC [g C (kg EOC)$^{-1}$] by (A) biochemical quality of EOC or (B) biochemical quality and initial apparent decomposition of EOC. Results of correlations used for validation of previous regressions and of the cross-validation method used for calibration of regressions: R^2 Coefficient of determination, R_a^2 adjusted determination coefficient, F number of factors in PLS regression. All regressions were significant at the 0.001 probability level. C carbon concentration [g C (kg DM)$^{-1}$], WSC water-soluble carbohydrates [g (kg DM)$^{-1}$], SOL hot-detergent-soluble organic matter [g (kg DM)$^{-1}$], CEL cellulose [g (kg DM)$^{-1}$], LIC lignin [g (kg DM)$^{-1}$], C_{3d} apparent decomposition in 3 days of incubation at 22 °C [g C (kg EOC)$^{-1}$]

Model for C_{pot} estimation		Variables in the regression	Coefficients					Validation / Calibration (n = 30)		
			a0	a1	a2	a3	a4	R^2	R_a^2	F
Validation of current indicators of C_{pot} (Lashermes et al., 2009)										
A	D2	SOL, LIC	226	0.2	1.2			0.53	0.51	2
	D3	SOL, LIC	262	0.3	1.0			0.71	0.70	2
B	D2	SOL, CEL, LIC, C_{3d}	445	0.5	-0.2	0.7	-2.3	0.95	0.94	3
	D3	SOL, CEL, LIC, C_{3d}	446	0.4	-0.2	0.7	-1.6	0.95	0.94	3
Recalibration of current indicators of C_{pot} (Lashermes et al., 2009)										
A	D2	CEL, LIC	783	-1.4	0.7			0.75	0.73	2
	D3	CEL, LIC	760	-1.5	0.7			0.77	0.75	2
B	D2	CEL, LIC, C_{3d}	928	-1.2	0.3	-2.8		0.95	0.94	2
	D3	CEL, LIC, C_{3d}	895	-1.2	0.3	-2.6		0.95	0.94	3
Reconstitution current indicators of C_{pot} (Lashermes et al., 2009)										
A	D2	C, LIC	924	-1.9	2			0.92	0.91	2
	D3	C, LIC	900	-2	2			0.92	0.91	2
B	D2	C, CEL, LIC, C_{3d}	946	-0.5	-0.8	0.7	-2.4	0.96	0.95	2
	D3	C, CEL, LIC, C_{3d}	916	-0.5	-0.9	0.7	-2.2	0.96	0.95	2

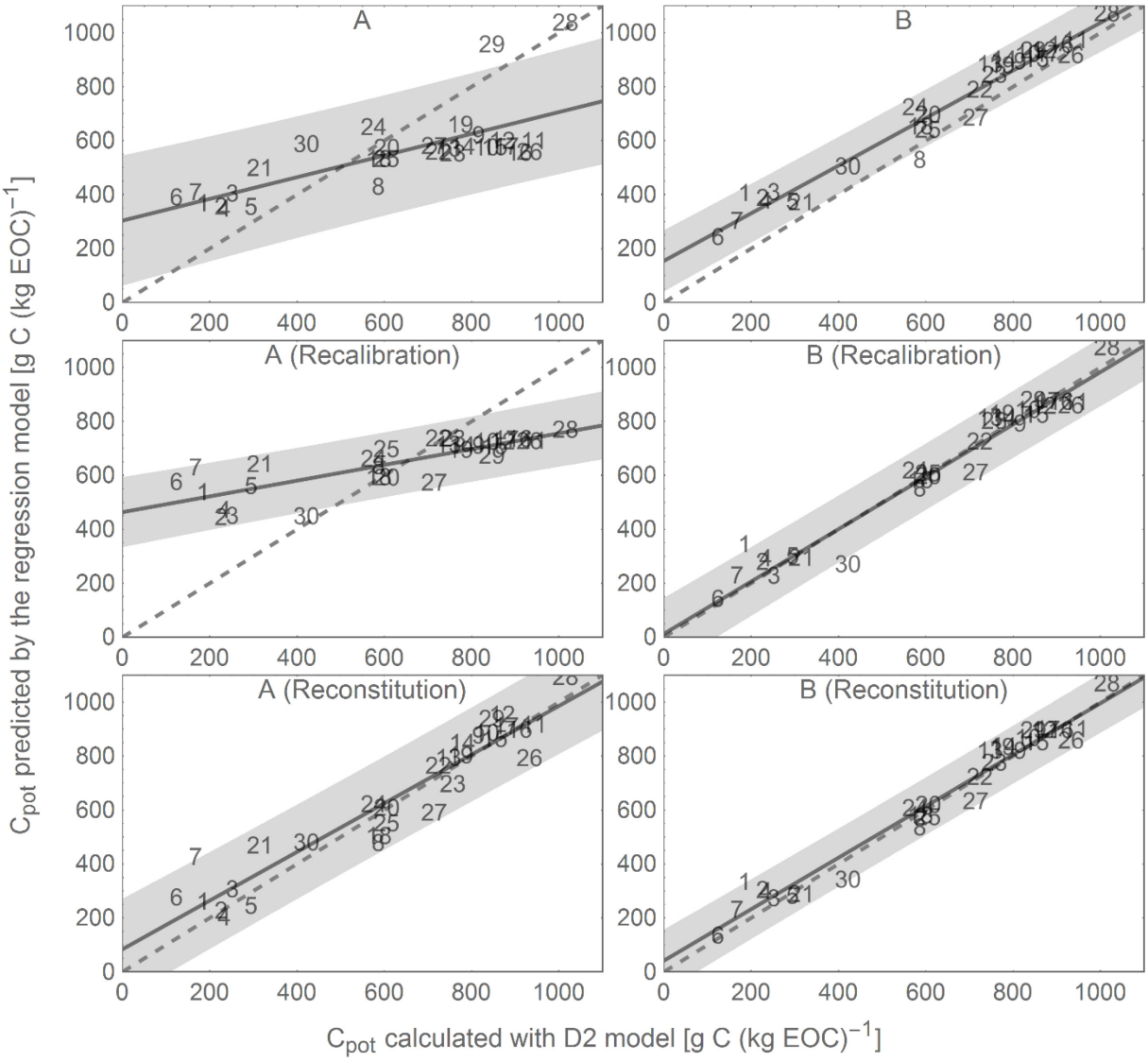

Figure 27 C_{pot} calculated with D2 model versus current indicators: (A) 226 + 0.2 SOL + 1.2 LIC, and (B) 445 + 0.5 SOL – 0.2 CEL + 0.7 LIC – 2.3 C_{3d}, their recalibrations: (A) 783 – 1.4 CEL + 0.7 LIC, and (B) 928 – 1.2 CEL + 0.3 LIC – 2.8 C_{3d}, and their reconstitutions: (A) 924 – 1.9 C + 2 LIC, and (B) 946 – 0.5 C – 0.8 CEL + 0.7 LIC – 2.4 C_{3d}. Relation given as linear regression (black line) ± 0.95 % PI (shaded) towards the background of the 1:1-relation (dashed grey line). Different numbers indicate different EOC samples by the EOC sample ID (n = 40).

A more detailed comparison of model-based calculations and indicator-based estimations of C_{pot} revealed similar average differences for each EOC group (Table 24). In general, indicator-based estimations following regression A and B overestimated the actual C_{pot}-values, which were calculated following the D2 model. Especially the current indicator (regression type B), overestimated C_{pot} of plant residues, urban composts, and farm fertilisers. An overestimation also occurred for the particularly high C_{pot} of char, but in this case constituted a relatively slight bias. The C_{pot} of digestates was over- and underestimated by both regressions. Regression B underestimated C_{pot} of solid separation digestates, whereas regression A further underestimated the separation digestate of organic waste and the digestate of maize & slurry.

Table 24 Calculated C_{pot} with D2 model C_{pot} and estimations by the current indicator (B) $I_{pot} = 445 + 0.5$ SOL – 0.2 CEL + 0.7 LIC – 2.3 C_{3d} and the reconstituted indicator (A) $I_{pot} = 942 – 1.9$ C + 2 LIC. Means (least square means) of groups and types of 30 EOC samples.

Group	Type	Sample	C_{pot}	I_{pot} (A)	I_{pot} (B)
				g C (kg EOC)$^{-1}$	
Plant residue	Wheat straw	1	185	260	406
		2	226	227	387
	Rape straw	3	252	306	409
	Rye straw	4	234	202	377
	Sorghum litter	5	295	245	374
	Sorghum stubble	6	124	276	243
	Sorghum coarse root	7	168	426	303
	Sorghum fine root	8	586	478	528
	Mean (plant residue)		**266**	**311**	**376**
Urban compost	Organic waste compost	9	816	876	894
		10	834	886	922
	Municipal solid waste compost	11	943	916	972
		12	870	954	935
	Organic waste & municipal solid waste compost	13	747	794	884
		14	779	849	900
		15	853	863	903
		16	910	898	955
	Green waste compost	17	879	910	922
	Mean (urban compost)		**840**	**894**	**923**
Farm fertiliser	Farmyard manure	18	587	504	653
	Farmyard manure (rotted)	19	774	802	879
		20	606	608	696
	Cattle slurry	21	315	468	371
	Mean (farm fertiliser)		**530**	**559**	**604**
Digestate	Organic waste	22	724	764	788
	Organic waste (separated)	23	757	696	844
	Maize silage	24	576	621	724
	Maize silage & Slurry	25	605	551	640
	Maize silage & Slurry (solid)	26	933	792	917
	Cofermentation (solid)	27	714	590	685
	Mean (digestate)		**718**	**669**	**766**
Char	Biochar	28	1015	1093	1072
	HTC-wheat straw	29	846	938	935
	HTC-poplar wood	30	422	480	504
	Mean (char)		**761**	**837**	**837**

4.4 Discussion

4.4.1 Comparison of biochemical quality for energy crop residues, digestates, and char

The results showed fundamental differences in biochemical quality between energy crop residues, digestates, and char (Table 20). As energy crop residues were crude materials for decomposition, they still contained high amounts of water-soluble carbohydrates, whereas digestates or manures in general and char were virtually free of water-soluble carbohydrates. Singly hydrothermally carbonised char still contained substantial amounts of water-soluble carbohydrates, if the duration and magnitude of pressure influence on the source material was insufficient for a complete pyrolysis (Bruun et al., 2012). In example HTC-poplar wood, which was pyrolysed at 200 °C for 20 min still contained water-soluble carbohydrates, whereas HTC-wheat straw, which was pyrolysed at 200 °C for 3 h solely contained a marginal amount of them. A second fundamental difference between energy crop residues, digestates, and char occurred in the portion of lignin. While lignin portions were less than 100 g (kg DM)$^{-1}$ for plant residues (Jensen et al., 2005), they were more than 100 g (kg DM)$^{-1}$ for manures (composts, farm fertilisers, and digestates) (Lashermes et al., 2009). Therefore, lignin accumulates during microbial conversion of plant residues and EOC, as easily available carbon compounds like water-soluble carbohydrates had been preferentially metabolised (Cotrufo et al., 2013). The highest portions of lignin showed char, especially biochar and HTC-wheat shoot. The longer exposure of materials to high temperatures and pressure during hydrothermal carbonisation increases the portion of lignin in EOC (Libra et al., 2011), and provides an explanation for the higher portion of lignin in HTC-wheat straw than in HTC-poplar wood. In this case, it might be more suitable to characterise this portion as 'lignin-like', as insolubility in sulfuric acid rather indicated the crystallisation of lignin and numerous aliphatic carbon compounds (Keiluweit et al., 2010) than a sole enrichment of lignin in pyrogenic organic matter (Schmidt et al., 2011).

The evaluation of biochemical quality by the fibre fractionation into NDF, ADF, and ADL (Van Soest and Wine, 1967) as it is used for the used for determination of forage digestibility (following the German standard VDLUFA, 1976), and the calculation of SOL, HEM, CEL, and LIC as differences of these fractions (Lashermes et al., 2009) was restricted, as neutral detergent fibre (NDF) was lower than acid detergent fibre (ADF) for certain EOC types of urban composts, digestates, but also char. This resulted in apparently negative portions of hemicellulose (HEM) for these EOC types (De Neve et al., 2003, Sullivan et al., 2004, Lashermes et al., 2009). As the neutral detergent solution contains a higher diversity and portion of applied detergents and chelators than the acid detergent solution, the lower NDF-value might be caused by substances of EOC, i.e. pectin and tannin, which are insoluble in acid detergent solution, but soluble in neutral detergent solution, and implies a sequential analysis of neutral and acid detergent solution for the determination of ADF to calculate HEM (Van Soest et al., 1991). According to previous investigation on biochemical indicators for C_{pot} (Lashermes et al., 2009), this investigation did not apply a sequential analysis of ADF and therefore excluded the fractions of SOL and HEM from further consideration.

The EOC group of digestates could not be differentiated from other groups of manures, as manures commonly were of low C/N-ratios and low concentrations of water-soluble carbohydrates. Irrespective of the microbial conversion path (digestion, fermentation or composting), manures could be differentiated as a whole from the unprocessed plant residues.

The thermally processed char could be biochemically distinguished from both plant residues and manures. Char thereby constituted a new biochemical quality, combining high C/N-ratios with a large proportion of organic matter, which was insoluble in sulfuric acid and is commonly referred to as lignin. In contrast to microbial conversion, which causes a successive release of carbon, pyrolysis involves the successive concentration of carbon, as the concentration of O, H, N, and S decreases, while functional aromatic units are being condensed (Usman et al., 2015). Charred EOC therefore inheres a unique biochemical quality in the spectre of organic soil compounds.

4.4.2 Decomposition of energy crop residues, digestates, and char

Decomposition largely varied between energy crop residues, digestates, and char; but also within groups of EOC (Figure 26). While litter, stubble, and coarse root of Sorghum showed similar courses of EOC-induced CO_2-release than straw of different crop species, fine roots released less carbon from the beginning on and finally left more C_{pot} after decomposition in soil (Table 21). Manures, especially urban composts and digestates released less carbon in decomposition than plant residues and could therefore be characterised as more stable and more persistent in soil.

The course of EOC-induced CO_2-release for digestates depended on the type of source material (substrate), as in the first three days of incubation it was less for digestates of organic waste than for the ones of maize silage and slurry. Further differences occurred in subsequent decomposition stages, when easily available carbon compounds supposedly became scarce. Although digestates, urban composts, and farm fertilisers could not be biochemically distinguished, certain EOC types largely differed in decomposition, i.e. digestates of maize silage and slurry released less carbon than slurry, whereas the unseparated digestate of organic waste released more carbon than the compost of organic waste. Therefore the characterisation of biochemical quality might remain insensitive to important biochemical alterations, which assumedly occur during microbial conversion. Furthermore, digestates, which had undergone a solid phase separation and appeared in solid or semifluid consistence, released less carbon in decomposition than unseparated digestates of similar substrates (Grigatti et al., 2011), i.e. the solid digestate of maize und slurry released least carbon of all digestates. This way, digestates spanned a range of total carbon release within the range of farmyard manures and even composts, particularly confirming the broad range from total stability in soil (Kolar et al., 2008) to a substantial carbon release, which is less than for plant residues (Moorhead et al., 1987, Sanger et al., 2011). Although high initial decomposition rates might be associated with soil organic carbon priming, especially for the unseparated digestates, none of the observed digestates released more carbon in incubation experiments, than initially

applied. Therefore no further evidence for the appearance of negative C_{pot}-values (Bernal and Kirchmann, 1992) due to excessively positive soil organic carbon priming could be provided.

Decomposition of char depended on the pyrolysis method. Biochar, which is produced in dry pyrolysis at high temperature, released very low amounts of carbon in decomposition, whereas hydrothermal char, which was produced at low temperatures, released more carbon in decomposition than biochar (Malghani et al., 2013, Bamminger et al., 2014). Furthermore, a negative priming effect of biochar had become apparent since the 21st day of incubation (Maestrini et al., 2015). However, thermal stabilisation effectively reduced accessibility of EOC to microbial decomposition (Bruun et al., 2012): While 80 % of wheat straw carbon were released in incubation experiments, solely 15 % of HTC-wheat straw carbon could be released by microbial decay. Furthermore, the total carbon loss of HTC-wheat straw was even lower than the total carbon loss of HTC-poplar wood, as wheat straw was exposed to hydrothermal carbonisation for a longer period of time, which increased the 'lignin-like' portion (Libra et al., 2011), decreased the portion of water-soluble carbohydrates (Bruun et al., 2012), and therefore decreased mineralisation of EOC and SOC priming (Bamminger et al., 2014). Although both microbial conversion and thermal conversion effectively reduced accessibility of EOC to microbial decomposition in incubation experiments, the pyrolysis of EOC inhered the highest potential to increase C_{pot}-values.

Parameters of biochemical quality and the course of EOC-induced CO_2-release were tightly correlated with each other, and EOC-induced CO_2-release in the early days of incubation was further correlated with the total carbon loss (Table 22). This supported previous regression analysis for an indicator of C_{pot} to be either based on biochemical quality parameters or based on both biochemical quality and carbon release within the initial 3 days of incubation (Lashermes et al., 2009). The inclusion of the carbon release in the first 3 days of incubation into an indicator remains a reasonable extension, as it was tightly correlated to total carbon release, but not significantly correlated to most of the biochemical quality parameters (Lashermes et al., 2009). However, the setup of an incubation experiment is enormously elaborate, whereas this initial carbon release into an indicator of C_{pot} should rather be replaced by suitable biochemical parameters. The portions of water-soluble carbohydrates and hemicelluloses were positively correlated to carbon release in the initial 3 days of incubation, but not applicable for regressions as water-soluble carbohydrates solely occurred in plant residues and partially in hydrothermal char, whereas the reliable determination of hemicelluloses remained methodologically fragile. Singly the portion of lignin, which was negatively correlated with EOC-induced CO_2-release since the 3rd day of incubation, constituted an alternative substitute. After the 3rd day of incubation the correlation between certain biochemical properties and EOC-induced CO_2-release successively increased (Jensen et al., 2005). The total carbon loss was not correlated with the initial nitrogen concentration in EOC samples (Lashermes et al., 2009), as the initial mineral N supply compensated for lower N concentrations in EOC samples. Contrary to previous investigation (Lashermes et al., 2009), the total carbon loss was positively correlated with the total carbon concentration in EOC samples,

whereby carbon concentration was based on dry matter in this investigation and on organic matter in previous investigation. Therefore, the total carbon concentration firstly expressed the portion of organic matter in dry matter, which could be decreased due to soiling, and secondly expressed the gravimetric proportion of carbon in relation to other organic elements, i.e. O, H, N, S, and P. The likewise significant positive relation between total carbon loss and initial C/N-ratio indicated the more release of carbon from carbon-rich compounds like water-soluble carbohydrates, hemicelluloses, and celluloses than from nitrogen-rich compounds like proteins or lignin. Proteins might rather be used by microbes for the built-up of microbial biomass, whereas the accessibility of lignin requires the presence of energy-rich carbon compounds (Cotrufo et al., 2013). Therefore, total C concentration, the C/N-ratio, and the portions of water-soluble carbohydrates, hemicellulose, and cellulose were negatively correlated with C_{pot}, while the initial N concentration and the portion of lignin were positively correlated with C_{pot}.

4.4.3 Biochemical indication of C_{pot}

The current indicator $I_{pot} = 445 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d}$ (Lashermes et al., 2009) predicted the C_{pot} for the main groups of EOC groups, which included plant residues and several manures, but not energy crop residues, digestates, or even char. The hypothesis that C_{pot} of these different groups of EOC, which had undergone totally different conversion processes, could not be predicted by a single indicator, was contradicted in several ways. Firstly, the high adjusted coefficient of determination (0.95) for the current indicator I_{pot}, which could neither be increased by a recalibration nor by a reconstitution of the underlying regression, contradicted the assumption that different EOC groups require separate biochemical indicators.

The current proposal for an indicator, which is singly based on the parameters of stepwise chemical digestion $I_{pot} = 226 + 0.2\ SOL + 1.2\ LIC$ reached relatively low coefficients of determination in validation for the main groups of EOC (0.46) (Lashermes et al., 2009) and in validation for these emerging groups of EOC (0.51) (Table 23). This lack of accuracy could be resolved by a reconstitution of the underlying regression, as the biochemical parameter SOL was exchanged into the total initial carbon (C) concentration of EOC. The reconstituted indicator proposal $I_{pot} = 924 - 1.9\ C + 2\ LIC$ reached an adjusted determination of 0.91, therefore provided a similar accuracy like regressions, which include the carbon release in the initial 3 days of incubation as parameter, and provided a second contradiction of the assumption that different EOC groups require separate biochemical indicators. However, the negative relation between total initial C concentration and C_{pot} was not plausible for all EOC groups: Digestates, which were characterised by low C/N-ratio and low C concentrations particularly left as much C_{pot} after decomposition as char did, which in contrast was characterised by high C/N-ratio and high C concentrations. Despite this unsteadiness between total initial C concentration and C_{pot}, the reconstituted regression proposal for an indicator based on singly biochemical properties predicted quite well the high C_{pot}-values both of EOC samples, irrespective of different groups (Figure 27, lower left graph). One reason for this might be the compensation by another biochemical property,

for example the 'lignin-like' fraction, which was apparently higher for char than for digestates but was integrated into regression with the opposite algebraic sign than the total C concentration.

Both regression types (A and B in Table 24), with and without initial EOC-induced CO_2-release as predictive parameter overestimated the actual C_{pot} for each group of EOC, except for digestates. While the regression type B (with initial EOC-induced CO_2-release as predictive parameter) in general overestimated C_{pot}, the regression type A equally underestimated C_{pot} of certain plant residues, farm fertilisers, and digestates. The general overestimation of regression type B might have occurred, as it had been calibrated for biochemical quality parameters based on organic matter (Lashermes et al., 2009), whereas in this investigation they were based on dry matter, which consisted of organic matter and crude ash. Therefore, in this investigation the calculated portion of SOL contained crude ash and was increased, which might have increased the estimation for C_{pot} in regression type B. However, both regression types provided estimations of C_{pot} for each group of EOC at similar accuracy, which finally contradicted the assumption that different EOC groups require separate biochemical indicators.

4.5 Conclusion

The emerging importance of extraordinary EOC, i.e. energy crop residues, digestates, and char in recent agricultural development came up with the necessity of a revaluation of the current indicator of C_{pot}. It was hypothesised that due to fundamental differences in biochemical quality of plant residues, manures, and char, separate indicators of C_{pot} would be required each of these EOC groups. Indeed, drastically differences in biochemical quality occurred, while the course of EOC-induced CO_2-release and C_{pot} partially overlapped between different EOC groups. Char was characterised by high C/N ratio, while digestates were characterised by low C/N ratio. Furthermore, char showed the largest portion of acid detergent organic matter (referred to as 'lignin-like'), which was even much larger than of digestates. Digestates revealed a broad spectre of C_{pot}, depending on the fermented substrate, the use of a solid-phase separated after fermentation, and the final consistence of the digestate. Although digestates particularly reached as much C_{pot} as char after decomposition. In spite of fundamental differences in biochemical quality, the current indicator $I_{pot} = 445 + 0.5\ SOL - 0.2\ CEL + 0.7\ LIC - 2.3\ C_{3d}$, ($R^2 = 0.96$, $n = 30$) was able to predict C_{pot} for all groups of EOC and could be complemented by a further regression for all groups of EOC, which was exclusively based on biochemical indication: $I_{pot} = 924 - 1.9\ C + 2.0\ LIC$, ($R^2 = 0.92$, $n = 30$), both contradicting the hypothesis.

5 Nitrogen enhances initial decomposition and persistence of plant residues in cultivated soils

5.1 Introduction

In agricultural, and more general terrestrial ecosystems, nitrogen (N) enrichment increases the magnitude of terrestrial carbon input from aboveground (litter) but decreases microbial respiration (microbial activity) and microbial biomass carbon (microbial growth), while the response of soil organic carbon stocks remains insignificant and difficult to predict (Liu and Greaver, 2010). Although increased aboveground litter input enhances carbon availability for microorganisms, nitrogen enrichment equally favours several processes, which either reduce carbon availability for microorganisms or directly inhibit microbial growth (Treseder, 2008), i.e. decreasing belowground carbon input (Treseder, 2004), decreasing ligninase activity (e.g. Waldrop and Zak, 2006), increasing production of melanoidins (Soderstrom et al., 1983, Fog, 1988), and processes, which are caused by a decrease of the soil pH (Vitousek et al., 1997) or even toxic osmotic potentials (Broadbent, 1965). However, the increasing magnitude of organic layer carbon and dissolved organic carbon in soil (Liu and Greaver, 2010) emphasizes the necessity of an increasing microbial decomposition and conversion of aboveground litter ('humification') into stable decomposer products (Cotrufo et al., 2013). In the long-term, soil organic carbon stocks are predicted to increase and not necessarily reach an equilibrium, if N is abundant and steadily accumulates in terrestrial ecosystems (Fontaine and Barot, 2005). In fact, soil organic carbon stocks are able to continuously increase in terrestrial ecosystems, as shown for old-growth forests (Zhou et al., 2006). Although agricultural ecosystems are exposed to N enrichment at large rates, decreasing carbon stocks were reported (Bellamy et al., 2005). This discrepancy in responses of soil organic carbon stocks between natural and agricultural terrestrial ecosystems, presumably refers to the totally different nitrogen loads in both terrestrial ecosystems. The influence of versatile N applications and loads on soil organic carbon stocks of cultivated fields therefore importantly issues agricultural SOC management.

Nitrogen fertilisation has outreached the application as ingredient of agricultural organic amendments since the invention, production and application of mineral N. Nitrogen fertilisation has been applied organically as farmyard manure since the Early Middle Ages in Europe. In the 18th century, the inclusion of legumes as forage crop into the crop rotation by Johann Christian Schubarth, Edler vom Kleefelde, provided N fertilisation via symbiotic N fixation for agricultural use. After the invention of the chemical N fixation the by Fritz Haber and Carl Bosch and its technical realisation in the 20th century, mineral N fertilisation uncoupled from carbon sources established. The international organic nitrogen fertilisation long-term field experiments (IOSDV) reproduce this historical development and examine the influence of organic and mineral N fertilisation on soil fertility at several European sites. They constitute a common crop rotation of summer cereal, winter cereal, and root crop, being fertilised (i) organically with farmyard manure or green manure, and (ii) with mineral N on successive levels.

However, the specific response of soil organic carbon stocks on the versatile N loads is difficult to measure in situ. Therefore N effects on transformation processes of recently applied EOC (e.g. straw) have been focused as key processes in agricultural ecosystem carbon cycling. Incubation experiments observe these key processes in vivo under standardised environmental conditions, whereby changes in density fractions or particle size fractions of SOC can be precisely detected after the homogeneous incorporation of straw and mineral N into a sample of soil. Mineral N supply thereby compensates for differences between C:N stoichiometry of straw and soil organic matter (Kirkby et al., 2011), increases SOC formation after straw addition ('humification efficiency'), and is proposed to sustain soil fertility beyond the single replacement of harvest N exports (Kirkby et al., 2013, 2014). Furthermore, incubation experiments allow for indirect determination of short-term N effects on straw decomposition by cumulative measurement of CO_2 release (identified as microbial activity) and periodical measurement of microbial biomass carbon (identifying microbial growth). In this way, N supply was found to progressively increase microbial activity after straw addition in the initial 60 days of incubation (Henriksen and Breland, 1999). Although microbial growth was equally increased by N supply (Henriksen and Breland, 1999), the specific carbon release of microbial biomass carbon (indicated as 'metabolic quotient' qCO_2) was decreased, or conversely the microbial carbon use efficiency (CUE) was increased by N supply (Manzoni et al., 2012b) and high litter N concentrations (Cotrufo et al., 2013). In a nutshell, N supply has been identified to enhance microbial accessibility and CUE of straw.

In contrast to short-term N effects on decomposition of recently applied EOC, effects of the soil fertilisation history (especially the N fertilisation history, Figure 4) on EOC decomposition have been contentiously issued so far. Most authors support the concept, that soil fertilisation effects on decomposition of recently applied organic amendments (EOC) are neglectable (Fauci and Dick, 1994, Hadas et al., 1996, Langmeier et al., 2002, Stark et al., 2008, Nett et al., 2012). The application of organic fertilisers has been shown to increase microbial growth in the long-term (Houot and Chaussod, 1995, SalinasGarcia et al., 1997, Gunapala and Scow, 1998, Kandeler et al., 1999), to induce alterations in microbial community structure (Dambreville et al., 2006, Ruppel et al., 2007, Stark et al., 2008), and to increase enzyme-activity in soil (Dick et al., 1988, Carpenter-Boggs et al., 2000). In spite of these responding biological soil properties, the influence of the soil fertilisation history on decomposition of crop residues has been declared to be insignificant (Nett et al., 2012). In the IOSDV experiments, soil fertilisation history has been demonstrated to affect physicochemical soil properties, as nitrogen concentrations in fertilised soils were increased (Spiegel et al., 2010) and density-fractions of soil organic matter particularly altered (Winkelmann et al., 2006). This might imply alterations in C:N stoichiometry of soil organic matter, which might further influence effects of mineral N supply on crop residue decomposition (Finn et al., 2015). However, C:N stoichiometry of soils is assumed to be constant (Kirkby et al., 2011) and might only vary within a small range. There is still a lack of evidence for soil fertilisation history effects on decomposition of recently applied EOC to evaluate interactions with mineral N supply and the substitution of both in order to increase SOCS and N immobilisation.

This investigation compares effects of mineral N fertilisation and organic amendments in the long-term with effects of mineral N supply in the short-term on straw decomposition in laboratory incubation, addressing to the following questions:

Does long-term organic amendment history affect straw decomposition?

Does soil fertilisation history affect straw decomposition?

How does mineral N supply affect straw decomposition and persistence in soil?

Does soil fertilisation history influence the effect of mineral N supply on straw decomposition?

How do fertilisation history, mineral N supply, and straw application affect microbial activity in soil?

How do fertilisation history, mineral N supply, and straw application affect microbial growth and microbial carbon use efficiency in soil?

Does mineral N supply influence N immobilisation during straw decomposition?

How do soil fertilisation history, mineral N supply and straw decomposition influence soil pH?

We collected soil samples from the IOSDV experiment in Berlin-Dahlem for a long-lasting incubation experiment with straw (representing plant residues), to study the influences of soil fertilisation history on straw decomposition, microbial activity, and microbial growth in the absence and presence of mineral N supply. Our main hypothesis was, that effects of mineral N supply and soil fertilisation history substitute each other, as both will initially increase decomposition and finally increase C_{pot} of straw.

5.2 Material and Methods

5.2.1 Incubation experiment

5.2.1.1 Soil and Site

The long-term field experiment IOSDV differentiates organic and mineral N fertilisation since 1984 (Figure 28). A crop rotation of winter cereal, potatoes, and summer cereal was implemented (visible as three fields alongside, Figure 28). Within each field, all combinations of organic fertilisation (farmyard manure (FYM), straw-green manure (SGM), and without organic manure (NON)) and four steps of mineral N fertilisation (0, 60, 110, and 160 kg N ha^{-1} y^{-1}, addressing to no, suboptimal, optimal, and excessive mineral N fertilisation, respectively) were realised in a split-plot design by three replications (blocks). The green-manure fertilisation had been conducted once in a crop rotation cycle as application of potato by-products and leaves of sugar beet, directly after potato cultivation.

In March 2014, after winter wheat cultivation in the northern field (lower left field at the IOSDV- field experiment, Figure 28), but before any mineral fertiliser was applied, topsoil (0-30 cm) was representatively collected and pooled from each replication of selected soil fertilisation treatments within this field. The soil selection contained the organic fertilisation variants (NON, FYM, and SGM) with and without mineral fertilisation. For straw-green manure fertilisation, all four mineral fertilisation steps were selected, while for farmyard manure fertilisation solely one treatment with excessive mineral fertilisation and one without mineral fertilisation were selected. All soils were sieved at field moisture in two steps by 5 mm, followed by 2 mm mesh width, and stored at 5 °C.

Contemporaneously to the soil collection, each selected field replication (n = 3) was sampled until 30 cm depth for a chemical soil analysis. Field samples and a subsample of each collected soil were analysed for pH-value (according to DIN 19684) with a pH-meter (WTW Multiline® IDS), mineral N concentration (according to DIN 19746), and further ground (Retsch® ball mill) to pulverised samples. The pulverised samples were analysed for total carbon (EN 15936) and total nitrogen concentrations (EN 16168), using elementary analysis (elementar® varioMAX®) after dry combustion (Dumas, 1831).

Out of the collected soils, five were chosen for an incubation experiment, containing the soil fertilisation history variants no nitrogen fertilisation (NON), straw-green manure without mineral N fertilisation (SGM), straw-green manure with optimal mineral N fertilisation by 110 kg N per hectare and year (SGM110), and straw- green manure with high mineral N fertilisation by 160 kg N per hectare and year (SGM160), and farmyard manure without mineral N fertilisation (FYM). Before incubation, the soils were stored for 10 days at 22 °C, to adapt soil microbial community to incubation conditions.

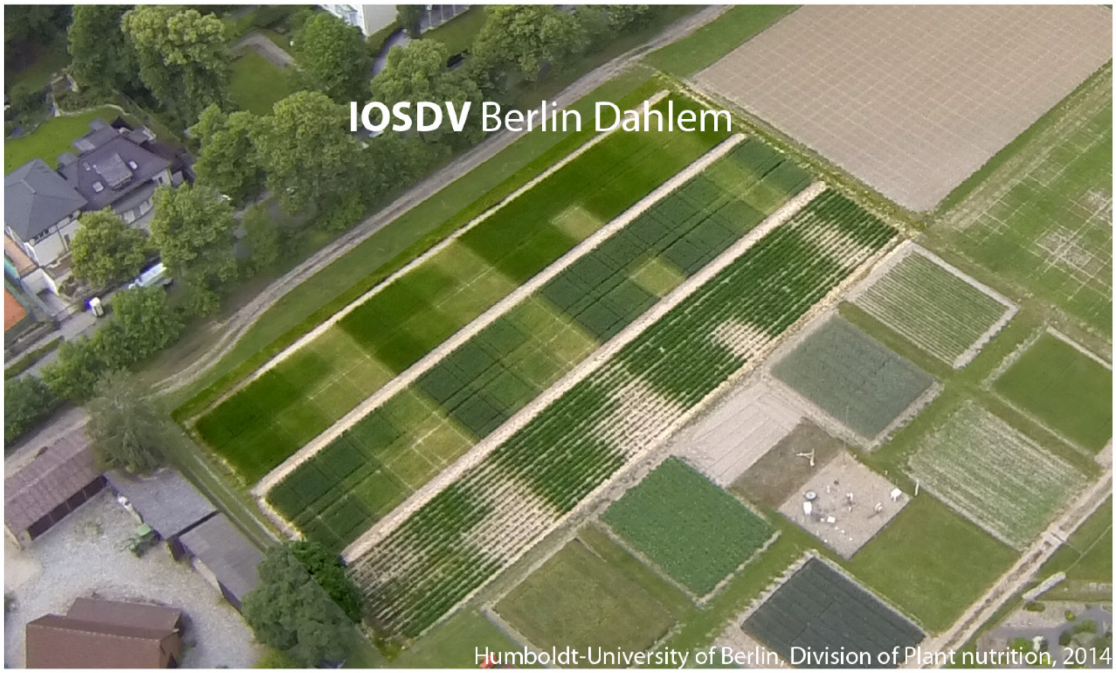

Figure 28 International organic nitrogen long-term field experiment (IOSDV) in Berlin Dahlem, set for analysis of the impact of organic and mineral N fertilisation on soil fertility. Photo of the field experiment with three fields (longitudinal from right to left) of potato, winter cereal, and summer cereal.

5.2.1.2 Setup of the incubation study

Soils of different soil fertilisation history (collected soils of IOSDV) were further treated with different recent fertilisation (straw addition and mineral N supply) and filled in small PE-containers, so called "soil columns". Straw, as standard EOC, was cut into pieces of 1 mm particle size by milling (RETSCH® SM 2000) and homogeneously mixed into soil at a rate of 400 mg EOC (100 g soil)$^{-1}$. According to Henriksen and Breland (1999), to half of the soil columns mineral N was supplied in order to reach a common level of 100 mg N (kg soil)$^{-1}$. The soil was compacted to 1.1 g cm^{-3} bulk density. Four PE-containers without soil were incubated as blanks. All treatments were replicated (n = 4), in total 64 soil

columns and 4 blanks were prepared (4 IOSDV treatments × 2 straw addition × 2 N supply × 4 replications + 4 blanks). At the start of incubation, soil water content was adjusted to 50 % of water holding capacity (ISO 16072), by adding 14.6 ml H_2O per 100 g soil. Incubation temperature was 22 °C. After incubation, subsamples of each soil column were extracted with 0.0125 M $CaCl_2$ for measurement of pH-value (DIN 19684) and a spectrometric determination of mineral N (ammonium and nitrate) concentrations (DIN 19746).

5.2.1.3 Measurement of CO_2 release during the incubation study

The soil columns were placed in closed jars with 100 ml 0.2 M NaOH at the bottom, absorbing the mineralised CO_2, which was released from the soil columns between two measuring dates. The absorbed CO_2 was precipitated as $BaCO_3$ through the addition of 10 ml 1.5 M $BaCl_2$ solution and measured by titration with 0.4 M HCl and phenolphthalein as indicator. Measurement dates were 1, 3, 7, 14, 21, 35, 56, 77, 98, 120, 162, and 217 days after start of incubation. The microbial activity was measured as difference between solved CO_2 in jars with soil columns and blanks. The apparent decomposition of straw was calculated as difference between evolved CO_2 from soil columns with and without straw. Kinetics for both, microbial activity and straw decomposition were calculated by successively summing up the EOC-induced CO_2-release between two subsequent measurement dates. The microbial activity and apparent decomposition of straw were indicated as cumulative carbon release in g C $(vessel)^{-1}$ and g C $(g\ EOC)^{-1}$, respectively. This approach is named "apparent" decomposition, as it integrates SOC priming and EOC mineralisation.

5.2.1.4 Measurement of microbial biomass carbon and nitrogen after 3 days of incubation

After three days of incubation, one replication of each soil column that had been additionally prepared, was destructively analysed for microbial biomass carbon and nitrogen (n = 1), following fumigation-extraction methodology (ISO 14240-2) with three laboratory repetitions: Six subsamples (15 g soil) were taken, whereof three joined the fumigation with chloroform to induce a lysis of microbial tissues and cells, before all six subsamples were extracted with 60 ml 0.5 M K_2SO_4. Carbon determination in the extracts was conducted in *elementar* liquiTOC®, following (ISO 8245). Additionally, microbial biomass nitrogen was determined in the extracts, using colorimetry (DIN 19746) after persulfate oxidation (Cabrera and Beare, 1993). As microbial biomass carbon and nitrogen are solely partially extractable, the detected amounts of both were divided by 0.45 (Joergensen, 1996) and 0.54 (Joergensen and Mueller, 1996), respectively.

Microbial carbon use efficiency (CUE) was calculated as metabolic quotient (qCO_2) at the 3rd day of incubation, and indicated as mg C h^{-1} $(g\ MBC)^{-1}$:

$$qCO_2 = \frac{C_{3d} - C_{1d}}{45h\ MBC}$$

C_{3d} is EOC-induced CO_2-release in the initial 3 days of incubation, C_{1d} is EOC-induced CO_2-release in the 1st day of incubation, *45 h* is the period between C_{1d} and C_{3d}, and *MBC* is microbial biomass carbon.

5.2.2 Statistical analysis and modelling

5.2.2.1 Statistical analysis of soil properties before incubation

The analysis of soil fertilisation history effects on soil pH, total C and N concentrations, C/N-ratio of soil, mineral N concentration, and pH-value was conducted as one-way-ANOVA of the single fix factor soil fertilisation history. Overall means of each soil fertilisation treatment were calculated as unweighted least square means. The effect of the fix factor soil fertilisation history was tested with an F-test and consecutively distinguished by pairwise comparisons, using Tukey-HSD-test (alpha at the 0.05 probability level). This statistical analysis was conducted in SAS® 9.2.

5.2.2.2 Mathematical model for decomposition of straw

The parallel first order kinetic model (Manzoni et al., 2012a) was fitted to all apparent straw courses of EOC-induced CO_2-release, using Marquardt-Levenberg algorithm. Parallel first-order kinetics describe decomposition of two biodegradable carbon pools in incubation experiments:

$$C(t) = \alpha\, C_s\left(1 - e^{-k_1 t}\right) + (1-\alpha)\, C_s\left(1 - e^{-k_2 t}\right)$$

where *C (t)* is the EOC-induced CO_2-release in g C (g EOC)$^{-1}$, C_s is the potentially biodegradable pool in g C (g EOC)$^{-1}$, k_1 and k_2 are two different mineralisation rate constants in days^{-1}, α marks the portion of the biodegradable pool C_s which is decomposed at mineralisation rate k_1, and *t* is the time of incubation in days. All model fits were conducted in Wolfram *Mathematica*® 10.2.

5.2.2.3 Calculation of C_{pot}

The course of EOC-induced CO_2-release was modelled by the D2 model (details in section 2.2.5.1 on page 31). Potential residual organic carbon (C_{pot}) was determined for straw in each soil sample (N = 8) as described in chapter 3.2.3.2 on page 67.

5.2.2.4 Microbial activity, microbial biomass carbon and nitrogen, and metabolic quotient

For each measurement date, the cumulative carbon release (referred to as microbial activity) was analysed by a linear mixed model in a full factorial design of three fix factors:

$$y_{ijk} = \mu + a_i + b_j + c_k + ab_{ij} + bc_{jk} + ac_{ik} + abc_{ijk} + \varepsilon_{ijk}$$

Where a_i is the effect of soil fertilisation history, b_j is the effect of short-term straw addition, c_k is the effect of mineral N supply, while ab_{ij}, bc_{jk}, ac_{ik}, and abc_{ijk} are all corresponding interactions. The models were set up under consideration of the co-variance structure and Kenward-Roger degrees of freedom approximation (n = 4, N = 64).

For the 3rd day of incubation, the microbial biomass carbon (MBC) and the metabolic quotient (qCO_2) were analysed by the linear mixed model as applied for cumulative carbon release, whereof the interaction of all three effects abc_{ijk} had been removed (n = 1, N = 16):

$$y_{ijk} = \mu + a_i + b_j + c_k + ab_{ij} + bc_{jk} + ac_{ik} + \varepsilon_{ijk}$$

The according microbial biomass nitrogen was considered to calculate the microbial C/N-ratio. The microbial C/N-ratio was analysed for treatments with straw addition by a reduced linear mixed model (n = 1, N = 8):

$$y_{ijk} = \mu + a_i + c_k + ac_{ik} + \varepsilon_{ik}$$

Where a_i is the effect of soil fertilisation history and c_k is the effect of mineral N supply, while ac_{ik} is the corresponding interaction. In each of the applied models, the effects and interactions of fix factors were tested with an F-test and consecutively distinguished by pairwise comparisons, using Tukey-HSD-test (alpha at the 0.05 probability level). This statistical analysis was conducted in SAS® 9.2.

5.2.2.5 Mineral N concentration and pH-value after incubation

After incubation, the mineral N concentration and pH-value of all soil columns were analysed by linear mixed models in a full factorial design of three fix factors:

$$y_{ijk} = \mu + a_i + b_j + c_k + ab_{ij} + bc_{jk} + ac_{ik} + abc_{ijk} + \varepsilon_{ijk}$$

Where a_i is the effect of soil fertilisation history, b_j is the effect of short-term straw addition, c_k is the effect of mineral N supply, while ab_{ij}, bc_{jk}, ac_{ik}, and abc_{ijk} are all corresponding interactions. The models were set up under consideration of the co-variance structure and Kenward-Roger degrees of freedom approximation (n = 4, N = 64). In each model, the effects and interactions of fix factors were tested with an F-test and consecutively distinguished by pairwise comparisons, using Tukey-HSD-test (alpha at the 0.05 probability level). This statistical analysis was conducted in SAS® 9.2.

5.3 Results

5.3.1 Chemical soil properties in the IOSDV long-term field experiment Berlin Dahlem

Soil fertilisation history, lasting for 30 years, significantly affected chemical soil properties in the plough horizon of the sandy loam in Berlin-Dahlem (Table 25). Straw-green manure fertilisation (SGM) increased total C concentration, especially in combination with mineral N fertilisation. The combination of SGM and mineral N fertilisation came up with the highest SOC concentration of 0.7 per cent of dry matter. Thereby, mineral N fertilisation even decreased the C/N-ratio of soil in the intensively fertilised variants SGM110 and SGM160. Both straw-green manure and mineral N fertilisation significantly increased the N concentration in soil, indicating N enrichment over long periods of time. In March 2014, highly variable mineral N concentrations could be observed, that were lowest under omitted fertilisation (NON) and for treatments with farmyard fertilisation (FYM), in detail 1 and 2.3 mg N (kg soil)$^{-1}$), and highest for straw-green manure with a mineral N fertilisation of 110 kg N ha^{-1} year^{-1} (SGM110), in details 6.4 mg N (kg soil)$^{-1}$. In case of straw-green manure and mineral N fertilisation of 110 kg N ha^{-1} year^{-1}, a significantly lower soil pH-value of 5.76 was reached than for farmyard manure fertilisation (pH 6.03). In contrast to SGM fertilisation, the FYM fertilisation did not differ from NON in any soil

property. In a nutshell, soil fertilisation history effects on chemical soil properties were solely apparent for straw-green manure and a further complementation by mineral N fertilisation.

Table 25 Effect of soil fertilisation history on chemical soil properties of a sandy loam in Berlin-Dahlem: C_{total} soil carbon concentration, N_{total} soil N concentration, C/N-ratio of soil, N_{min} mineral N concentration, and pH-value of soil solution. Means of sampled field replications (n = 3): Different lower case letters behind means (x-z) indicate significant (Tukey-Test, $\alpha = 0.05$) differences between means of different soil fertilisation history. NON omitted fertilisation, SGM (60-160) straw-green manure (with mineral N fertilisation at 60-160 kg N ha^{-1} $year^{-1}$), FYM (160) farmyard manure (with mineral N fertilisation at 160 kg N ha^{-1} $year^{-1}$).

Soil fertilisation history	C_{total} [% DM]	C/N	N_{total} [mg (kg soil)$^{-1}$]	N_{min} [mg (kg soil)$^{-1}$]	pH
NON	0.50 x	13.1 x	380 x	1.0 x	5.86 xyz
SGM	0.64 y	12.5 xy	513 y	3.6 y	6.06 xyz
SGM60	0.70 y	12.3 xy	566 yz	4.6 y	5.89 xy
SGM110	0.70 y	11.9 y	594 z	6.4 z	5.76 x
SGM160	0.70 y	12.2 y	575 yz	5.7 xyz	5.85 y
FYM	0.56 xy	12.8 xy	436 xy	2.2 xy	6.03 z
FYM160	0.62 xy	12.4 xy	496 y	2.3 y	5.94 yz

5.3.2 Influence of soil fertilisation history and mineral N supply on straw decomposition, microbial activity, and microbial growth

5.3.2.1 Decomposition of straw

Straw decomposition depended on both mineral N supply and soil fertilisation history (Figure 29). Both influenced the course of EOC-induced CO_2-release specifically for different periods of time during incubation. Considering the three different stages of carbon availability (as described in Figure 21), soil fertilisation history especially affected straw decomposition in the intermediate (since the 3rd day of incubation) and the final decomposition stage, whereby a scarcity of easily available carbon for microorganisms was assumed (see left graph in Figure 29).

Since the 3rd day of incubation, the straw-induced CO_2-release had been lower in the soil of omitted fertilisation (NON) than in fertilised soils, irrespective of fertilisation type. According to the CIs of the fitted decomposition models, the EOC-induced CO_2-release further differed in soils of different fertilisation types, whereas the higher EOC-induced CO_2-release was simulated in the soil of SGM110 fertilisation than in the soils of SGM and FYM fertilisation. Subsequently, EOC-induced CO_2-release continued at higher rates until the end of the incubation experiment for the soil of omitted fertilisation (NON) than for fertilised soils, whereby it further continued at higher rates for soils of SGM110 fertilisation than for soils of SGM and FYM fertilisation. Therefore an intersection of all differing

courses of EOC-induced CO_2-release occurred. This intersection occurred after different periods of incubation. The intersection of the courses of EOC-induced CO_2-release in soils of different soil fertilisation history occurred at the 98th day of incubation, whereas the intersection of the courses of EOC-induced CO_2-release in the soil of omitted fertilisation and in each of the soils of SGM110, SGM, and FYM fertilisation occurred later, within the period from 150th to 175th day of incubation. At the end of the incubation experiment the most carbon of initially added straw was lost in the soil of omitted fertilisation, which was simulated to be higher than in the soils of SGM and SGM110 fertilisation. The EOC-induced CO_2-release was predicted to be lowest in the soil of SGM110 fertilisation. Furthermore, the fitted models predicted for a longer period of incubation the straw-induced CO_2-release to be higher in soil of omitted fertilisation than in all fertilised soils. Therefore, fertilisation finally reduced the total carbon loss of straw.

If mineral N was supplied in incubation experiments (see right graph in Figure 29), the straw-induced CO_2-release occurred at higher rates than without N supply in the first 21 days of incubation, irrespective of fertilisation type. However, differences between EOC-induced CO_2-release in soils of different soil fertilisation history equally occurred in the presence of mineral N supply, but the intersection of the courses of EOC-induced CO_2-release occurred after 21 days of incubation, which was much earlier than in the absence of N supply. At the 56th day of incubation, the simulated course of EOC-induced CO_2-release differed between soil fertilisation treatments: The highest EOC-induced CO_2-release occurred in the soil of FYM fertilisation, which was even more than in the soil of omitted fertilisation, whereas the least EOC-induced CO_2-release was simulated in soils of the SGM and SGM110 fertilisation. After the 56th day of incubation, the rate of EOC-induced CO_2-release rapidly decreased, irrespective of fertilisation type. Finally, more carbon was lost in the soil of FYM fertilisation than in soils of omitted, SGM or SGM110 fertilisation. Therefore, the long-term effect of omitted fertilisation on EOC-induced CO_2-release in decomposition of straw was reversible by a single mineral N supply, while the long-term effect of different types of organic fertilisation could not be compensated in incubation experiments by mineral N supply.

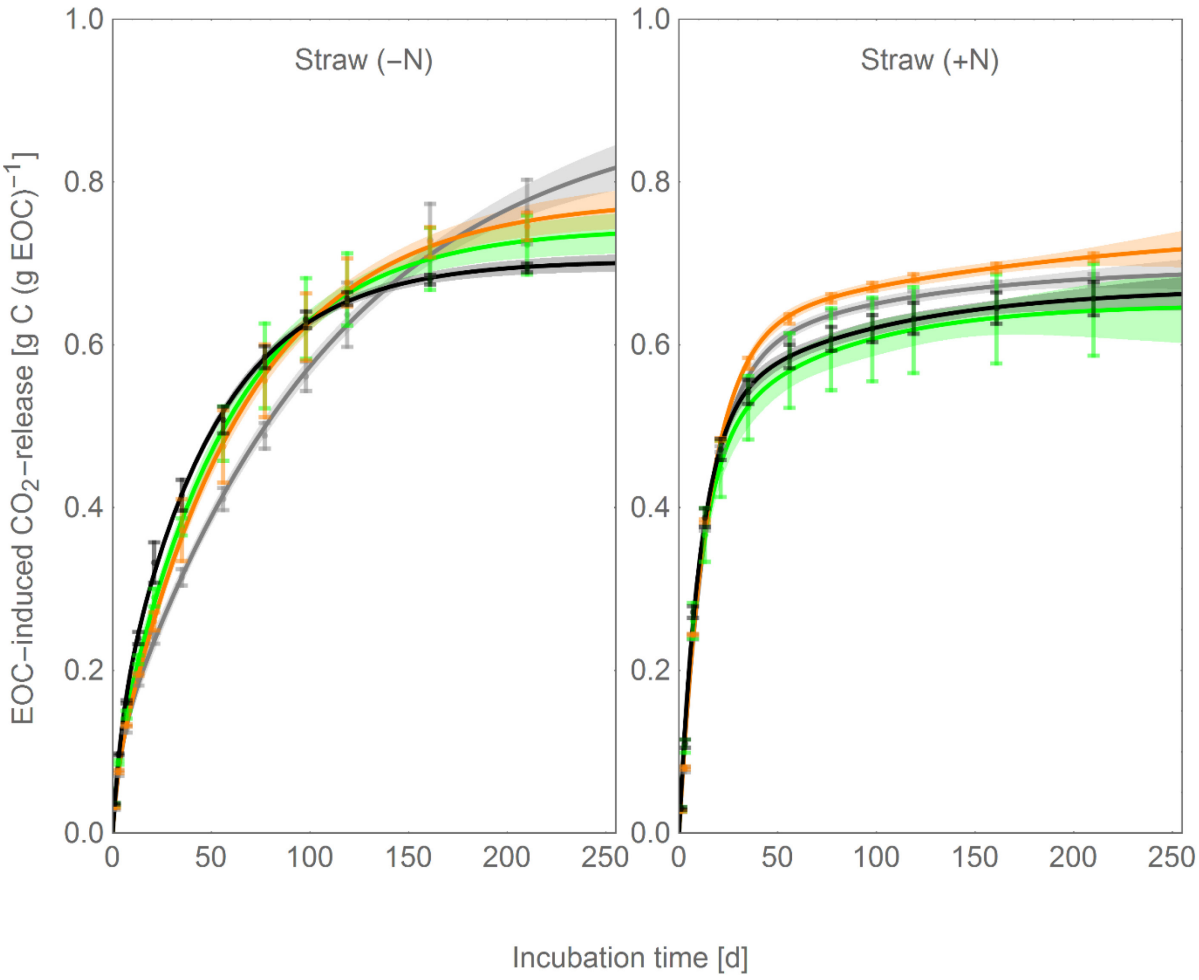

Figure 29 Apparent decomposition of straw with (right) and without (left) mineral N supply in soils of different soil fertilisation history: No nitrogen fertilisation (grey), Farmyard manure fertilisation (orange), Straw-green manure fertilisation (green), Straw-green manure and 110 kg N ha^{-1} mineral N fertilisation (black). Means ± standard deviation (n = 4), D2 model ± 95 % CI (shaded).

Across soil fertilisation treatments, the influence of mineral N supply on the course of EOC-induced CO_2-release was specific for different periods of incubation (Figure 30). Initially, from start of the incubation experiment until the 3rd day of incubation, no effect on the modelled straw-induced CO_2-release was apparent, as CIs did not show any differences. Subsequently, the straw carbon was released at higher rates in the presence than in the absence of mineral N supply, as the modelled straw-induced CO_2-release in both soils successively differed (Henriksen and Breland, 1999). Implications for persistence of straw require longer periods of incubation than 30 to 50 days. Even before the 56th day of incubation, the maximum difference had been reached and both courses of EOC-induced CO_2-release approached until they intersected 120 days after incubation. At the end of the incubation experiment and presumably for infinite periods of incubation, even more carbon was lost from straw in the absence than in the presence of mineral N supply, according to the modelled course of EOC-induced CO_2-release.

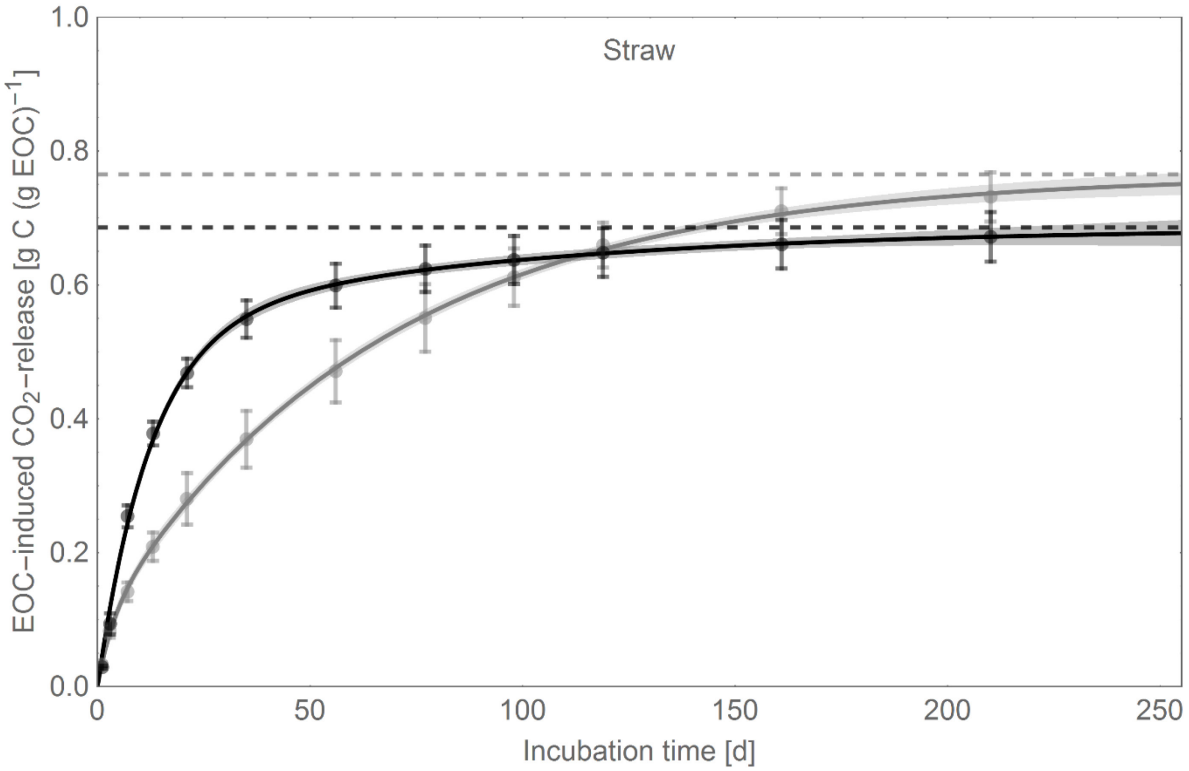

Figure 30 Apparent decomposition of straw with (black) and without (grey) mineral N supply at a rate of 100 mg N (kg soil)$^{-1}$, given as overall mean across soil fertilisation history ± standard deviation (n = 16), D2 model ± 95 % CI (shaded), and model limit (dashed).

The C_{pot} of straw, which is assumed to remain in soil after decomposition, depended on either mineral N supply or in case of omitted N supply on fertilisation type (Table 26). If both mineral N supply and soil fertilisation history were omitted, the lowest C_{pot} -value of straw, in detail 100 g (kg EOC)$^{-1}$ was obtained. Fertilisation of the soils increased the C_{pot}-values of straw, irrespective of amendment type. In case of SGM110 a C_{pot} -value of 300 g (kg EOC)$^{-1}$ was reached, which was more than in case of FYM and even could not be increased by mineral N supply. Across all soil fertilisation treatments, the average C_{pot} -value of straw was significantly higher in the presence of mineral N supply than it was in the absence of mineral N supply, overall means of 310 and 230 g (kg EOC)$^{-1}$, respectively. Mineral N supply increased C_{pot} of straw in the soil of omitted fertilisation from 100 up to 310 g (kg EOC)$^{-1}$. Therefore both mineral N supply and long-term soil fertilisation increased C_{pot} of straw.

Table 26 C_{pot} of straw for the different soil fertilisation treatments NON omitted nitrogen fertilisation, SGM (110) straw-green manure (with optimal mineral N fertilisation), and FYM farmyard manure fertilisation given as D2 model ± 95 %-CI (n = 4), and modelled mean across all soil fertilisation treatments for the variants with (+ N) and without (– N) mineral N supply given as D2 model ± 95 %-CI (n = 16).

Soil fertilisation history	Mineral N supply	
	– N	+ N
	C_{pot} [g C (kg EOC)$^{-1}$]	
NON	100 ± 63	310 ± 41
SGM	260 ± 29	350 ± 64
SGM110	300 ± 12	330 ± 40
FYM	220 ± 30	250 ± 138
Mean (mineral N supply)	230 ± 22	310 ± 40

5.3.2.2 Microbial activity

The cumulative carbon release of a certain soil characterises the metabolic activity of the aerobe carbon-heterotroph microorganisms in it and is also referred to as microbial activity. The influence of soil fertilisation history, mineral N supply, and straw addition on microbial activity depended on the period of incubation (Table 27). Soil fertilisation history affected microbial activity until the 56th day of incubation, whereas the effects of N supply and straw addition (and the interaction of both) on microbial activity were significant at the 0.001 probability level until the end of the incubation experiment. In spite of the insignificant soil fertilisation history effect at the end of the incubation experiment, the interaction of soil fertilisation history and straw addition on microbial activity was significant. The interaction of all three main effects on microbial activity was significant until the 56th day of incubation.

Table 27 Results of the F-test for the main effects and interactions of soil fertilisation history, N supply, and straw addition on microbial activity after different periods of incubation: * (**, ***) indicate significance at the 0.001 (0.01, 0.05) probability level.

Effect / Interaction	Period of incubation		
	3 d	56 d	210 d
Soil fertilisation history	***	**	
N supply	***	***	***
Straw addition	***	***	***
Soil fertilisation history × N supply	***	***	
Soil fertilisation history × Straw addition	***	*	**
N supply × Straw addition	***	***	***
Soil fertilisation history × N supply × Straw addition	**	***	

Soil fertilisation history, mineral N supply, and straw addition influenced microbial activity specifically for different periods of incubation (Table 28). Three days after start of the incubation experiment, neither an effect of soil fertilisation history nor an effect of mineral N supply was apparent for microbial activity in cultivated soil. Everything changed, when straw had been added. In this case a significant increase in cumulative carbon release was indicated in each of the soils. However, higher cumulative carbon release was found for the ones, which were fertilised with straw-green manure than for the ones in which soil fertilisation history was omitted. In contrast, such a difference was not apparent for soils which were fertilised with farm yard manure and the ones of omitted fertilisation. If no mineral N was supplied in incubation experiments, mineral N fertilisation in the long-term increased straw-induced CO_2-release (compare 39.5 with 35.8 mg C (soil column)$^{-1}$ for SGM110 and SGM, respectively). If mineral N was supplied in incubation experiments, the cumulative carbon release could be increased in both soils, which were fertilised with straw-green manure (SGM and SGM110) to a common value. Across all soil fertilisation treatments, the addition of straw increased cumulative carbon release from < 1 to 34.1 mg C (soil column)$^{-1}$, whereas mineral N supply further increased EOC-induced CO_2-release to 38.4 mg C (soil column)$^{-1}$.

After 56 days of incubation, more carbon was released from soils, which were fertilised with straw-green manure than from soils, which were fertilised with farmyard manure or in which soil fertilisation history was omitted. The mineral N supply decreased cumulative carbon release across all soil fertilisation treatments from 9.7 to 7.7 mg C (soil column)$^{-1}$, whereas straw addition increased cumulative carbon release to 198.1 mg C (soil column)$^{-1}$ or to 247.2 mg C (soil column)$^{-1}$, if it was combined with N supply.

After 210 days of incubation, the combination of straw addition and N supply led to less cumulative carbon release than the straw addition alone.

Table 28 Influence of soil fertilisation history (H), mineral N supply (R), and straw addition (EOC) on microbial activity after different periods of incubation. Means of cumulative carbon release (n = 4). Different lower case letters behind means (a-f) indicate significant (Tukey-Test, α = 0.05) differences among the interaction H × R × EOC; different lower case letters behind means (x-z) indicate significant (Tukey-Test, α = 0.05) differences among H: NON omitted fertilisation, SGM (110) straw-green manure (with mineral N fertilisation), FYM farmyard manure fertilisation; different lower case letters behind means (α-γ) indicate significant (Tukey-Test, α = 0.05) differences among the interaction R × EOC (recent fertilisation): + *N* N supply, + *Straw* Straw addition.

Soil fertilisation history	Recent fertilisation				Mean (soil fertilisation history)
	– Straw		+ Straw		
	– N	+ N	– N	+ N	
	Cumulative carbon release [mg C (soil column)$^{-1}$]				
3 days of incubation (referred to as initial straw decomposition stage)					
NON	0.8a	0.4a	30.2b	31.7b	15.8x
SGM	1.0a	1.1a	35.8c	43.8e	20.4y
SGM110	0.8a	1.2a	39.5d	45.2e	21.7z
FYM	0.6a	0.8a	30.8b	32.9b	16.3x
Mean (recent fertilisation)	0.8α	0.9α	34.1β	38.4γ	
56 days of incubation (referred to as intermediate straw decomposition stage)					
NON	8.3b	5.6a	172.5d	249.5f	109.0x
SGM	10.5bc	8.5b	206.8e	235.9f	115.4xy
SGM110	11.4c	9.5bc	214.4e	243.8f	119.8y
FYM	8.6b	7.1ab	198.7de	259.8f	118.6y
Mean (recent fertilisation)	9.7α	7.7β	198.1γ	247.2δ	
210 days of incubation (referred to as final straw decomposition stage)					
Mean (recent fertilisation)	23.1α	19.5β	315.7γ	288.26δ	

5.3.2.3 Microbial growth and CUE at 3rd day of incubation

Three days after start of the incubation experiment, microbial biomass carbon largely varied between soils with and without straw addition, but also between soils to which mineral N was supplied and those without mineral N supply (Table 29). Straw addition and N supply significantly affected microbial growth. Straw addition increased the magnitude of microbial biomass carbon to the threefold of cultivated soils, whereas N supply increased microbial biomass carbon from 182.5 to 236.7 µg C (g soil)$^{-1}$. The effect of soil fertilisation history could not be significantly determined (F-test, P = 0.054).

Table 29 Influence of soil fertilisation history, mineral N supply, and straw addition on microbial growth after 3 days of incubation. Means of microbial biomass carbon (n = 1). NON omitted fertilisation, SGM (110) straw-green manure (with mineral N fertilisation), FYM farmyard manure fertilisation; + *N* N supply; different lower case letters behind means (α-β) indicate significant (Tukey-Test, α = 0.05) differences among the effect straw addition: + *Straw* Straw addition.

Soil fertilisation history	Recent fertilisation				Mean (soil fertilisation history)
	– Straw		+ Straw		
	– N	+ N	– N	+ N	
	Microbial biomass carbon [µg C (g soil)$^{-1}$]				
NON	45.2	125.0	268.5	266.3	176.3
SGM	71.2	143.8	317.6	377.6	227.6
SGM110	95.6	152.4	310.5	357.3	229.0
FYM	64.0	141.1	287.5	329.8	205.6
Mean (recent fertilisation)	69.0	140.6	296.0	332.7	
Mean (straw addition)		104.8α		314.0β	

The metabolic quotient qCO_2 (conversely referred to as carbon use efficiency *CUE*) significantly responded on straw addition, the interaction straw addition × N supply, and the interaction straw addition × soil fertilisation history (Table 30). Effects of N supply and soil fertilisation history could not be significantly determined (F-test, P = 0.32 and P = 0.059, respectively). Straw addition increased qCO_2 from 1.1 to 16.5 mg C h^{-1} (g MBC)$^{-1}$, indicating less efficient carbon use after straw addition. Although the interaction straw addition × N supply was significant, an influence of N supply on qCO_2 was neither significant with nor without straw addition. In case of straw addition, qCO_2 was significantly higher in the soil, which was fertilised with straw-green manure and mineral N, 19 mg C h^{-1} (g MBC)$^{-1}$, than in the soil, which was fertilised with farmyard manure, 14.1 mg C h^{-1} (g MBC)$^{-1}$, respectively.

For treatments with straw addition, the microbial biomass nitrogen and the microbial C/N-ratio were determined. Neither an effect of soil fertilisation history nor an effect of mineral N supply on the microbial C/N-ratio could be significantly determined (F-test, P = 0.07 and P = 0.8, respectively). The microbial community under straw addition could therefore be characterised by C/N-ratio 5.3 ± 0.65, which was apparently lower than C/N-ratio of soil (compare Table 25).

Table 30 Influence of soil fertilisation history (H), mineral N supply (R), and straw addition (EOC) on metabolic quotient after 3 days of incubation. Means of metabolic quotient (n = 1). NON omitted fertilisation, SGM (110) straw-green manure (with mineral N fertilisation), FYM farmyard manure fertilisation; different lower case letters behind means (α-β) indicate significant (Tukey-Test, α = 0.05) differences among the interaction R × EOC: + *N* N supply, + *Straw* Straw addition.

Soil fertilisation history	Recent fertilisation				Mean (soil fertilisation history)
	– Straw		+ Straw		
	– N	+ N	– N	+ N	
	Metabolic quotient qCO_2 [mg CO_2-C h^{-1} (g MBC)$^{-1}$]				
NON	2,3	0,1	15,1	17,1	8,7
SGM	1,7	0,5	14,9	18,4	8,9
SGM110	1,2	0,7	17,9	20,1	10
FYM	1,8	0,6	13,9	14,4	7,6
Mean (recent fertilisation)	1,7α	0,5α	15,4β	17,5β	

5.3.3 Influence of mineral N supply and straw decomposition and on soil properties

5.3.3.1 Mineral N concentration after the incubation experiment

After the incubation experiment, the mineral N concentration (N_{min}) was significantly affected by soil fertilisation history, N supply, straw addition, and all possible interactions, except the interaction of soil fertilisation history × straw addition (F-test, P = 0.56) (Table 31). After the incubation experiment, the N_{min}-concentrations in soils were higher than before incubation (compare Table 25), indicating mineral N mobilisation during the experiment. In cultivated soils, which were fertilised with straw-green manure (SGM, SGM110) the N_{min}-concentrations were highest. The mineral N supply, which aimed to adjust N_{min}-concentrations to the common level of 100 mg (kg soil)$^{-1}$ could be confirmed after the incubation experiment. The addition of straw decreased the N_{min}-concentrations after the incubation experiment, indicating mineral N immobilisation during straw decomposition in the incubation experiment. However, the magnitude of this decrease depended on mineral N supply and soil fertilisation history, as it was 5 mg N (kg soil)$^{-1}$ in the absence of mineral N supply, whereas it was 20 mg N (kg soil)$^{-1}$ in the presence of mineral N supply. In the absence of mineral N supply, N immobilisation was singly significant for the soil, which was fertilised with straw-green manure.

Table 31 Influence of soil fertilisation history (H), mineral N supply (R), and straw addition (EOC) on mineral N concentration after 210 days of incubation. Means of mineral N concentration (N_{min}) (n = 4). Different lower case letters behind means (a-f) indicate significant (Tukey-Test, α = 0.05) differences among the interaction H × R × EOC; different lower case letters behind means (x-z) indicate significant (Tukey-Test, α = 0.05) differences among H: NON omitted fertilisation, SGM (110) straw-green manure (with mineral N fertilisation), FYM farmyard manure fertilisation; different lower case letters behind means (α-γ) indicate significant (Tukey-Test, α = 0.05) differences among the interaction R × EOC (recent fertilisation): + *N* N supply, + *Straw* Straw addition.

Soil fertilisation history	Recent fertilisation				Mean (soil fertilisation history)
	– Straw		+ Straw		
	– N	+ N	– N	+ N	
	N_{min} [mg (kg soil)$^{-1}$]				
NON	13ab	94d	8a	73e	47w
SGM	32c	101d	20b	84e	59x
SGM110	37c	103d	33c	85e	64y
FYM	17ab	97d	20b	75e	52z
Mean (recent fertilisation)	25α	99β	20γ	79δ	

5.3.3.2 pH-value after the incubation experiment

After the incubation experiment, the pH-value was significantly affected by soil fertilisation history, N supply, straw addition, and all interactions, except the interaction fertilisation type × N supply × straw addition (F-test, P = 0.32) (Table 32), whereby the range of measured pH-values did not differ before and after the incubation experiment (compare with Table 25). The average pH-value of cultivated soils after incubation was 5.83 across all soil fertilisation treatments. Short-term N supply to cultivated soil did not affect this average pH- value. The addition of straw and its subsequent decomposition led to a higher pH-value 5.94, which was further increased to pH-value 6.02, when straw addition was combined with N supply.

After the incubation experiment, an influence of soil fertilisation history on soil pH was apparent, as the average pH-values of fertilised soils across the different short-term treatments (N supply, straw addition) significantly differed (Table 32, right column). In contrast to the effects of straw addition and mineral N supply, the effect of soil fertilisation history was specifically directed for each amendment. The long-term amendment of farmyard manure, for example, led to a higher pH-value (pH 6.03) than omitted fertilisation (pH 5.95). The long-term amendment of straw-green manure led to a lower pH-value (pH 5.84) than omitted fertilisation, which was even lowest for the long-term amendment of straw-green manure with mineral N fertilisation (pH 5.79).

Table 32 Influence of soil fertilisation history (H), mineral N supply (R), and straw addition (EOC) on pH-value after 210 days of incubation. Means of pH-value (n = 4). Different lower case letters behind means (x-z) indicate significant (Tukey-Test, α = 0.05) differences among H: NON omitted fertilisation, SGM (110) straw-green manure (with mineral N fertilisation), FYM farmyard manure fertilisation; different lower case letters behind means (α-γ) indicate significant (Tukey-Test, α = 0.05) differences among the interaction R × EOC (recent fertilisation): + *N* N supply, + *Straw* Straw addition.

Soil fertilisation history	Recent fertilisation				Mean (soil fertilisation history)
	– Straw		+ Straw		
	– N	+ N	– N	+ N	
	pH-value				
NON	5.92	5.86	5.98	6.05	5.95y
SGM	5.74	5.79	5.85	5.97	5.84x
SGM110	5.68	5.72	5.84	5.92	5.79w
FYM	5.99	6.00	6.01	6.15	6.03z
Mean (recent fertilisation)	5.83α	5.84α	5.93β	6.02γ	

5.4 Discussion

5.4.1 Influence of soil fertilisation history on chemical soil properties

The results showed an influence of straw-green manure fertilisation on chemical soil properties, whereas the fertilisation of farmyard-manure and additional mineral N did not alter either pH, total C concentration, or the C/N-ratio of soil (Table 25). Firstly, organic amendments were not normalised in the IOSDV experiment, wherefore straw-green manure amendments applied twice as much carbon to soil as farmyard manure (Kautz, 2005). In this way, the enormous carbon load of straw-green manure fertilisation caused the increase of total C concentration. Secondly, there are fundamental differences between both organic amendments: Straw-green manure constitutes plant residues, which are known to consist of a large C/N-ratio and therefore immobilise mineral N during decomposition (Kirkby et al., 2013), whereas farmyard manure constitutes microbial derived residues, which consist of a similar C/N-ratio to soil organic matter. Additional mineral N fertilisation obviously enhanced N immobilisation in soils, which were fertilised with straw-green manure, as total C and N concentrations in soil could be further increased and C/N-ratio of SOC decreased. This supports a substantial N enrichment in the soil over time due to immobilisation of mineral N. The immobilisation of mineral N thereby enabled the conversion of the enormous carbon load of straw-green manure into the soil organic carbon stock. An analysis of particulate organic matter revealed an increase in the easily accessible light fraction of soil organic matter for straw-green manure fertilisation, wherefore farmyard manure increased the less accessible heavy fraction of soil organic matter (Winkelmann et al., 2006). Farmyard manure fertilisation further led to a higher soil pH-value than straw-green manure and mineral N fertilisation.

Therefore, slight changes in soil physicochemical properties, which were not detected in this investigation might have been even more beneficial for sustaining SOCS than the detected changes of straw-green manure fertilisation. However, physicochemical soil properties did not enable reliable implications of N fertilisation on the development of SOCS, which motivated the measurement of microbial processes during decomposition of a common plant residue.

5.4.2 Influence of soil fertilisation history on straw decomposition

As soil fertilisation history affected chemical soil properties, the main question was if decomposition of straw might be equally influenced. In contrast to previous investigation (Nett et al., 2012), the results of the incubation experiment showed significant effects of soil fertilisation history on the course and the absolute magnitude of straw-induced CO_2-release, which was homogeneously mixed into the soil, irrespective of additional mineral N supply (Figure 29). As independently of mineral N supply the straw-induced CO_2-release was initially higher in the soil, which had been fertilised with straw-green manure than in the soil, which had been fertilised with farmyard manure, differences in mineral N availability between both soils might not have been the reason for the different courses of EOC-induced CO_2-release. In fact, soil fertilisation history influences the microbial release of enzymes into soil (Dick et al., 1988, Carpenter-Boggs et al., 2000) and the structure of microbial communities (Dambreville et al., 2006, Ruppel et al., 2007, Stark et al., 2008), whereupon the microbial communities adapt to soil fertilisation history. The soil, which was fertilised with straw-green manure, assumedly contained a microbial community, which was better adapted to straw as a substrate than the soil, which was fertilised with farmyard manure. This adaption might have accelerated straw decomposition and equally increased efficiency of microbial carbon use (CUE) in the soil, which was fertilised with straw-green manure. The intersection of both courses of EOC-induced CO_2-release and the higher total carbon loss of straw in the soil, which was fertilised with farmyard manure, imply a lower CUE than in the soil, which was fertilised with straw-green manure.

Another important effect of soil fertilisation history in general solely occurred in the absence of mineral N supply. The results showed, that the course of EOC-induced CO_2-release was lowest in the beginning of the incubation but highest in the end of the incubation, if both mineral N supply and soil fertilisation history were omitted (Figure 29). As in this case, the highest total carbon loss of straw occurred, the CUE was impliedly lowest due to scarcity of available N (Manzoni et al., 2012b, Cotrufo et al., 2013). The addition of mineral N suspended N scarcity for decomposition, brought the courses of EOC-induced CO_2-release into line, and therefore compensated for omitted fertilisation.

In the presence of mineral N supply, each of the courses of EOC-induced CO_2-release was accelerated, whereas the intersection of them occurred in an earlier period of incubation than it was the case in the absence of mineral N supply. Although effects of different types of soil fertilisation history could not be suspended, all courses of EOC-induced CO_2-release were similarly modified by mineral N supply. Across the magnitude of differently fertilised soils (Figure 30), mineral N supply influenced the course

of EOC-induced CO_2-release since the 3rd day of incubation, whereby an acceleration (Henriksen and Breland, 1999) was followed by a deceleration, resulting in less total carbon loss. The reason for this deceleration might be increased CUE due to increased N availability (Manzoni et al., 2012b, Cotrufo et al., 2013).

Mineral N supply in laboratory incubation and soil fertilisation in the long-term both increased C_{pot} and therewith persistence of a common plant residue (e.g. straw) in soil, implicitly enabling the growth of soil organic carbon stocks (SOCS). Mineral N supply in incubation experiments or mineral N fertilisation in the long-term equally increased C_{pot}, and therefore partially substituted each other.

5.4.3 Influence of soil fertilisation history and mineral N supply on microbial activity

The effects of soil fertilisation history and mineral N supply on straw decomposition are derivations of effects on microbial activity in cultivated soils after straw incorporation. The results of an F-test showed, that the effect of soil fertilisation history on microbial activity disappeared at the end of the incubation experiment, whereas mineral N supply and straw addition enduringly influenced microbial activity (Table 27). This was concretised, as in the first three days of incubation, effects of soil fertilisation history and mineral N supply on microbial activity solely appeared in soils, into which straw had been incorporated before incubation (Table 28). The application of straw provided large amounts of fresh substrates to soil microorganisms, which in turn respond with an increased activity. Both mineral N supply and soil fertilisation obviously alleviated the microbial access of straw and further increased microbial activity. This relation turned during the incubation and similar to most terrestrial ecosystems, mineral N supply to cultivated soils finally decreased microbial activity (Liu and Greaver, 2010), irrespective of straw addition. However, in the beginning of the incubation the microbial response on mineral N supply depended on the contemporaneous addition of straw (Henriksen and Breland, 1999), which rather implied enhanced total CUE due to alleviated microbial access (Manzoni et al., 2012b, Cotrufo et al., 2013) than an inhibition of microbial growth (Treseder, 2008) in cultivated soils. An effect of soil fertilisation history on microbial activity in cultivated soil was apparent after 56 days of incubation, whereby the higher microbial activity in soils, which were fertilised with straw-green manure and mineral N might be due to higher amounts of the easily accessible light fraction of particulate organic matter (Winkelmann et al., 2006). Contrary to many terrestrial ecosystems (Liu and Greaver, 2010), a decrease in microbial activity due to mineral N fertilisation in the long-term could not be verified for cultivated soil, although an accumulation of undecomposed plant residues equally reveals a lack of microbial activity. Therefore, we assume the microbial community in soils of agricultural use to be adapted to high rates of N enrichment.

5.4.4 Influence of microbial growth and microbial carbon use efficiency at 3rd day of incubation

The results showed an increased microbial growth due to straw addition in the beginning of the incubation experiment (Table 29). Such a substrate-induced growth was expectable to appear and largely explained substrate-induced microbial activity. Contrary to many terrestrial ecosystems (Treseder, 2008,

Liu and Greaver, 2010) microbial growth positively responded on mineral N supply. Laboratory incubations do not account for N effects on plant growth and carbon supply to soil (Henriksen and Breland, 1999) – implications for ecosystem responses remain difficult. Increased microbial growth could be explained by increased microbial access of straw (Cotrufo et al., 2013), which in turn occurred to be limited by mineral N availability. Equally, soil fertilisation could be identified to increase microbial growth, whereby the measured microbial biomass carbon revealed the highest magnitude for soil, which was fertilised with straw-green manure and mineral N. This supported the assumption, that the microbial community of this soil had already been adapted to straw addition and mineral N supply, and therefore could better make it accessible. Microbial growth in cultivated soils therefore positively responded on a periodical N enrichment over long periods of time, which was in contrast to many terrestrial ecosystems (Treseder, 2008, Liu and Greaver, 2010).

The carbon use efficiency (CUE) in the beginning of the incubation was largely decreased by the addition of straw (compare Table 30). Without the addition of straw, the metabolism of microorganisms maintained essential vital functions (maintenance metabolism), whereas the addition of straw induced microbial growth and therefore a metabolic shift to a less efficient energetic mode (growth metabolism) (Campbell et al., 1999). Although N supply did not significantly influence CUE, the means revealed a contemporaneous tendency of increased CUE in cultivated soil but decreased CUE of newly added straw. Therefore, a stress reaction of microorganisms on eventual toxic N levels (Broadbent, 1965) could not be verified in cultivated soils for this rate of mineral N supply. If CUE of newly added straw had actually been decreased in this period of incubation, this would have rather indicated increased microbial growth metabolism due to enhanced microbial access of straw than a microbial stress reaction, and therefore might have implied a decreased CUE in the entire period of incubation. The response of CUE on the interaction of straw addition and soil fertilisation history provided further evidence for the adaption of microbial communities to this substrate, whereby the lower CUE in the soil, which was fertilised with straw-green manure and mineral N similarly revealed increased microbial growth due to enhanced microbial access of straw, compared to the soil, which was fertilised with farmyard manure.

The virtually constant C/N-ratio of microbial biomass, which was even lower than C/N-ratio of soil constituted a substantial need of available nitrogen to (i) make plant residues and soil organic matter accessible for microorganisms and to (ii) enable microbial growth. Therefore N supply in incubation experiments or via soil fertilisation history essentially enhanced microbial accessibility of substrates, increased microbial growth, and finally increased total efficiency of microbial carbon use. This implicitly increases the formation of SOC and reduces EOC-induced CO_2-release (Manzoni et al., 2012b, Cotrufo et al., 2013, Kirkby et al., 2013, 2014).

5.4.5 Mineral N immobilisation and pH-value at the end of the incubation

The results in Table 31 showed, that immobilisation of available mineral N depended on soil fertilisation history, as a significant immobilisation of mineral N was solely apparent for the soil, which was fertilised

with straw-green manure. As all other soils did not contain less mineral N after straw decomposition than cultivated soils in the end of the incubation experiment, the indifferent mineral N concentrations indicated the inability of the associated microbial communities to use N during straw decomposition as efficient as the microbial community that was adapted to the straw-green manure fertilisation.

Furthermore, the results verified the increment of the mineral N concentration in the soil to the common level of 100 mg (kg soil)$^{-1}$ by the initial mineral N supply, as there were finally no differences between the concentrations of mineral N in the variants to which mineral N initially was supplied. As there is evidence for high mineral N concentrations to inhibit microbial growth (Baath et al., 1981, Treseder, 2008), either directly due to altering osmotic potentials upon a toxic level (Broadbent, 1965) or indirectly due to decreasing soil pH (Vitousek et al., 1997), decreasing ligninase activity (e.g. Waldrop and Zak, 2006), and increasing condensation of easily available carbohydrates with nitrogenous compounds to less accessible melanoidins (Soderstrom et al., 1983, Fog, 1988), the mineral N supply at a constant level would have kept these unavoidable side effects constant, wherefore straw decomposition and N immobilisation could be independently discussed of any inhibition by mineral N supply. In the presence of mineral N supply, N immobilisation during straw decomposition occurred irrespective of fertilisation history. Mineral N supply increased the availability of mineral N for microorganisms, and microorganisms in turn used this increased external N availability for population growth and the formation of microbial products (Manzoni et al., 2012b, Cotrufo et al., 2013), compensating the difference between C/N- ratio of the added straw and soil organic matter (Kirkby et al., 2011, Kirkby et al., 2013). Therefore, N availability limited the conversion of straw into microbial biomass (Manzoni et al., 2012b), therewith SOC formation (Kirkby et al., 2014), implying a positive effect of N fertilisation on SOCS.

The results of the pH-measurement after incubation showed contrasting effects of short-term fertilisation and fertilisation history. In short-term, straw addition and its decomposition increased the soil pH, especially when straw addition was combined with mineral N supply. Straw decomposition encompasses both C and N mineralisation processes, whereby ammonification of organic N compounds and the further oxidation of ammonium to nitrate is known to decrease the soil pH-value (Ottow, 2011). Microorganisms in turn, are known to partially assimilate and therewith immobilise both forms of mineral N, nitrate and ammonium during decomposition (Ottow, 2011). As the initial addition of straw led to a significant immobilisation of mineral N, the soil pH-value was increased. The initial supply of mineral N further increased N immobilisation and therewith soil pH-value. However, incubation experiments postulate controlled environmental conditions, which do not include nitrate losses by leaching. In agricultural practice and long-term field experiments, nitrate leaching occurs and depletes cultivated soils of calcium and magnesium (Vitousek et al., 1997). Therefore, the positive effects of straw addition and N supply in the incubation could not be reproduced for the straw-green manure and mineral N fertilisation in the long-term field experiment (Table 25). Likewise in the incubation

experiment, straw-green manure and mineral N fertilisation had a negative effect on soil pH-value, whereas farmyard manure fertilisation positively affected soil pH-value.

5.5 Conclusion

Agricultural cropping systems differ from other terrestrial ecosystems as they are periodically loaded with N at higher rates via organic and mineral fertilisation. As the influence of N enrichment on SOCS in terrestrial ecosystems remains unclear, the main question of this investigation was, if N effects on microbial activity, microbial growth, microbial carbon use efficiency, N immobilisation, and soil pH during straw decomposition occur, and what implications for persistence of plant residues and maintenance of SOCS could be derived. For analytical regard, N effects were subdivided into short-term effects of recent mineral N supply and long-term effects of the soil fertilisation history. Fertilisation of straw-green manure and mineral N both caused a measureable N enrichment in soil over time. Irrespective of time-scale, N enrichment increased microbial activity in the beginning of straw decomposition and decreased microbial activity in the end of straw decomposition. Mineral N supply in coincidence with straw application accelerated decomposition and thereby compensated for omitted fertilisation. N enrichment increased microbial growth in the beginning of straw decomposition, which in turn increased N immobilisation and C_{pot} after decomposition in soil. Therewith N enrichment enhanced both decomposition and persistence of plant residues in cultivated soils. The explanation for this phenomenon has already been formulated as ‘humification efficiency’ (Kirkby et al., 2013) and microbial carbon use efficiency (Cotrufo et al., 2013), which both depend on N availability (Kirkby et al., 2014, Manzoni et al., 2012b). Moreover, N enrichment even increased the soil pH-value during straw decomposition in the closed system of the incubation experiments, whereas in the field experiment solely farmyard-manure amendments increased soil-pH, motivating further effort on the reduction of N leaching in cultivated fields to reproduce positive effects of straw and mineral N amendments on SOCS.

6 General Discussion and Conclusions

6.1 Opening: SOC formation – Impact of different environmental conditions and agricultural determinants of EOC persistence

In agriculture, EOC is applied to soil for compensation of SOC losses which are associated with crop production. Beyond the incorporation of plant residues, crop cultivation often requires organic fertilisation with plant-derived or microbial processed carbon sources to maintain SOC (Katterer et al., 2014). The transfer into the SOC pool still remains a complex process, which has roughly been described in two different pathways (Cotrufo et al., 2015): (i) the microbial path, on which water-soluble organic compounds are taken up by soil microorganisms and transformed into microbial products, contributing to the highly persistent mineral-stabilised SOC fraction and (ii) the physical transfer path, in which residues of cell wall constituents are incorporated into soil particles as a whole, contributing to the less persistent coarse fraction of SOC. Such direct measurement of SOC formation requires the isotopic labelling of EOC (Kirkby et al., 2014, Cotrufo et al., 2015), which is not feasible for all types of EOC. The indirect assessment of microbial decomposition and SOC formation as EOC-induced CO_2-release and C_{pot} has therefore served as methodological simplification in the comparative approach of different types and groups of EOC (Lashermes et al., 2009). Persistence of SOC is highly variable within and across different ecosystems, depending on physical and physicochemical soil properties (Schmidt et al., 2011). Additionally, biochemical properties of EOC largely vary between different types of plant-derived carbon sources, microbial processed carbon, and pyrogenic organic carbon significantly influencing the fraction of EOC, which is transferred into the SOC pool (Lashermes et al., 2009). Several investigations have focused on the C/N-ratio (Nicolardot et al., 2001), the aromaticity (Grabber and Coblentz, 2009), the concentration of different cell-wall constituents (Jensen et al., 2005) and the constitution of lignin (Talbot et al., 2012) as explanatory biochemical properties of EOC decomposition. However, the biochemical indication of the fraction, which is converted into SOC still remains a challenge, as biochemical parameters, which explain EOC decomposition in the initial 3 days of incubation have not been identified yet (Lashermes et al., 2009). Several research groups work on this field of 'humification' in incubation experiments, namely Jensen et al. (2005), Abiven et al. (2005), Lashermes et al. (2009), Manzoni et al. (2012b), Kirkby et al. (2014), and Cotrufo et al. (2015) - to mention a few, but each group defines an own measurement category (direct and indirect measures) for the 'humified' portion of EOC, uses different environmental parameter settings, and applies different mathematical models. Continuative research should therefore be intended to (i) prove the representativeness of the indirectly assessed C_{pot} for direct measurements of SOC formation, (ii) prove the comparability of decomposition in cultivated fields and controlled environmental conditions as applied in laboratory incubations, and (iii) work on the biochemical indication of the fraction of EOC, maintaining SOC in order to simplify, unify, and integrate incubation methodology and results.

In this study, it was attempted to cover (i) methodological insights into incubation experiments, focusing on environmental parameter settings, (ii) the biochemical indication of C_{pot} for different types of plant residues in energy-crop cultivation and more general different types of EOC, and (iii) long-term fertilisation effects on EOC decomposition. All three topics will be discussed in detail in the following discussion.

6.2 Is potential residual organic carbon (C_{pot}) robust towards different parameter settings in incubation experiments? (Chapter 2)

For the first question, the indirectly measured C_{pot} by EOC-induced CO_2-release was proven in its robustness towards different mathematical models, different environmental parameter settings and different C-availability. We found a close agreement (the best agreement) between measured and modelled data for the "D2" model which describes parallel first-order kinetics for decomposition of two biodegradable pools in EOC:

$$C(t) = \alpha\, C_s\left(1 - e^{-k_1 t}\right) + (1-\alpha) C_s\left(1 - e^{-k_2 t}\right)$$

This model allowed calculating the "potential residual organic C" (C_{pot}) of different types of EOC. Assuming a decomposition rate of humified carbon in the field of 0.02 $year^{-1}$ (according to Lashermes et al., 2009) and on the basis of a "biological active time" for decomposition in the field (which is mainly dependent on soil water content and soil temperature), the persistence of the EOC in soil under the environmental conditions in Berlin was calculated. To answer the question whether calculated C_{pot} is equivalent to humified EOC=SOC, we analysed the influence of soil type, incubation temperature and accessibility of EOC on decomposition / humification.

We incubated two EOC samples, straw and digestate, in four different soils. The 'Jena soil' was a silty clay loam containing 1.2 % organic carbon, 'Lobenstein soil' was a silt loam containing 2.8 % organic carbon, the 'Gießen soil' was a silt loam containing 1.2 % organic carbon, and the 'Berlin soil' was a sandy loam containing 1.6 % organic carbon (Table 1). C_{pot} was dependent on soil (Table 6). The soil effects differ depending on the type of EOC: For straw C_{pot} decreased in the order Berlin < Lobenstein < Gießen, Jena; for digestate C_{pot} decreased in the order Gießen (could not be determined) << Lobenstein < Berlin < Jena. According to the microbial efficiency – matrix stabilisation framework of EOC decomposition, which emphasizes the stabilisation of microbial products via adsorption onto mineral soil particles (Cotrufo et al., 2013), the soil effects could partly be explained by the increasing fine fraction of mineral soil particles in the order Berlin < Lobenstein < Gießen < Jena. The dependence of soil effects on the type of EOC was presumably due to other soil properties, like pH-value, the amount of available mineral N (Kirkby et al., 2014), and different SOC concentrations (Don et al., 2013), which influence mineralisation of EOC and the EOC-induced SOC-release (priming effect). At this point it remained unclear, why C_{pot} could not be determined in Gießen, but this was not issued in this thesis.

Another environmental parameter, which fundamentally differentiates decomposition in a cultivated field and in laboratory incubations is temperature. As laboratory incubation experiments keep a constant

temperature to identify the course of EOC-induced CO_2-release over long periods of time, different temperatures have been proposed to represent outdoor conditions (Sleutel et al., 2005). We compared maize stubble-induced CO_2-release at different temperature settings: (i) the constant temperature of 22 °C and (ii) 6°C for 161 days of incubation followed by 22 °C. We observed a progressive decrease of the rate of maize-stubble-induced CO_2-release due to cooling from 22 °C to 6 °C, which largely increased C_{pot}. However, this increase of C_{pot} was not robust towards subsequent rewarming to 22 °C, as microbial activity recovered and maize stubbles were decomposed to an equal stage like stubbles incubated at 22 °C since the beginning of incubation. During the incubation, the rate of EOC-induced CO_2-release more rapidly declined at 6 °C than at 22 °C. Low incubation temperatures have been suggested to even increase apparent N limitations of microbial activity due to a higher temperature-sensitivity of enzymes catalysing the breakdown of less accessible N-rich carbon compounds in soil (Karhu et al., 2014). Therefore, the temperature-sensitivity of EOC decomposition presumably depends on the availability of N from EOC for microorganisms, which largely varies among plant residues (Jensen et al., 2005). If temperature-sensitivity of EOC decomposition depended on the biochemical composition of EOC, the setting of a lower incubation temperature than 22 °C, as Sleutel et al. (2005) proposed, would rather decrease the comparability of different types of EOC in laboratory incubations than increase the comparability with outdoor-conditions in the cultivated field. High incubation temperatures might enhance enzymatic reactions, which are essential for microbial metabolism and the accessibility of high-molecular cell-wall constituents. We joined the common temperature of 22 °C, as it is proposed in (ISO 16072). Higher temperatures than 22 °C may occur in the topsoil for short seasonal times, the microbial community presumably remains less adapted to such a temperature range, and might expectedly respond less to a further temperature increase.

A second fundamental difference between decomposition in a cultivated field and decomposition in laboratory incubation is the C-availability. Carbon is supplied once in the beginning of the incubation at a rate (so called 'incubation ratio'), which is many times higher than in the field. The continuous input of carbon, which is typical for cultivated fields and natural terrestrial ecosystems, reduces limitations of easily-available carbon compounds for microorganisms. To operate as close as possible at cultivation-specific conditions, we collected the soil a few weeks before the incubation, kept the soil moisture until the setup of the experiment, decided for the incubation ratio of 400 mg C (100 g soil)$^{-1}$, which is lower than in previous investigations (Jensen et al., 2005, Lashermes et al., 2009), and tested the influence of a subsequent glucose application on decomposition of wheat shoot and maize digestate. Although we reduced the incubation ratio to 261 mg C (100 g soil)$^{-1}$ for wheat shoot, we observed a priming effect, which accounted for up to 20 % of wheat-shoot-induced CO_2-release. This result contrasted the equalisation of EOC-induced CO_2-release and mineralisation of EOC, used in Jensen et al. (2005) and Lashermes et al. (2009) for instance, as evidence was provided for a priming effect, which could not be neglected in incubation experiments. Consequently, we distinguished between 'EOC-induced CO_2-release' and 'mineralisation of EOC', as the EOC-induced CO_2-release contained both mineralisation of

EOC and EOC-induced mineralisation of SOC (referred to as 'SOC priming'). The priming effect mainly occurred in the initial 7 days of incubation. This implied a need for biochemical parameters of EOC, which explain the priming effect to reliably predict the initial EOC-induced CO_2-release by regression. The course of EOC-induced CO_2-release remained relatively robust towards the addition of glucose after 35 days of incubation for both types of EOC, the wheat shoot and maize digestate. The expectation, easily-available C might increase the microbial access to less-available C in maize digestate could not be confirmed. However, we observed a slight increase in EOC-induced CO_2-release and mineralisation of wheat shoot due to the addition of glucose. Therefore, it may be assumed, that the continuous C-supply to soil in cultivated fields substantially increases the mineralisation of plant residues, which is not reproducible for incubation experiments.

6.3 Is C_{pot} predictable by the biochemical composition of EOC? (Chapters 3 and 4)

The second question focused on the influence of biochemical properties of different plant residues and different types of EOC on C_{pot} in respect of an estimating equation (indicator) for relative differences in the fraction of the applied carbon, remaining and maintaining for SOC status of the soil. Firstly, we regarded plant residues, as fractionated into stubbles, coarse roots, fine roots, litter, and straw, from a field experiment, which was set to compare recently introduced species in energy-crop cultivation towards the background of 'well-known' C3-plants. We observed large differences in the biochemical composition of plant residues, depending on the crop residue type, the crop species respective cropping system, and the cultivation year (Table 12). Especially, the C/N-ratio was a parameter of biochemical quality, which largely varied in plant residues, whereby the concentration of nitrogen varied much more than the concentration of carbon. An even higher coefficient of variation was found for the concentration of water-soluble carbohydrates in plant residues. As both parameters are not included in the current indicator of potential residual organic carbon (Lashermes et al., 2009), they probably inhere a large potential for the biochemical indication of C_{pot} for plant residues from different crop species, which were harvested at different stages of phenological maturity. Comparing plant residues with other types of EOC, such as digestates, farm fertilisers, urban composts, and pyrogenic organic carbon, plant reisudes solely represented a slight portion of the biochemical variability of EOC.

One key issue of retracing carbon fluxes in agroecosystems was the fractionation of crop residues into different carbon compartments according to different plant organs and different modes of application to the soil (continuous or discontinuous deposition). The large biochemical differences between different fractions of the total crop residue, which we indicated as crop residue types, indicated a reasonable carbon compartmentation. Fine roots and litter were of the lowest C/N-ratio and solely contained marginal amounts of water-soluble carbohydrates. As both had been exposed to microbial decomposition before sampling and partially contained necrotic tissues, one might assume, the both of them might have been depleted of easily available carbon compounds. In fact, solely litter was depleted of water-soluble carbohydrates. The proportions of cell-wall constituents showed further differences

between crop residue types, as fine roots were of the highest lignin concentration and stubbles were of the highest cellulose concentration. High proportions of cellulose in stubble were expectable for a self-evident reason – the mechanical stability against tensile forces, whereas the lignification prepares fine roots for compressive forces in the soil.

The high annual variation solely partially enabled the biochemical differentiation of different crop species, which emphasizes the need for a biochemical indication of persistence instead of setting up constant coefficients for crop species in the humus balancing approach (Table 13). Crop residues of the legume pea contrasted the ones of winter wheat, oats, maize, sorghum, and Sudan grass (botanically the grass family), as they were of the lowest C/N-ratio and lowest hemicellulose concentration but of the highest lignin concentration. The results were related to Abiven et al. (2005), confirming high lignin concentrations in plant tissues of legumes. In contrast to legumes, winter cereal crop residues were of the largest hemicellulose concentration, for several possible reasons: Hemicelluloses are abundant in root mucilage (Rasse et al., 2005) but also an essential constituent of cell-walls (Strasburger et al., 2014). As pea is of repent figure, it may be assumed, that wheat, growing upright, requires more cellulose fibrils than pea and consecutively more hemicellulose to connect them. Thus, the cellulose concentration remained relatively constant among all crop species/ cropping systems, and differences in hemicellulose concentrations might rather indicate different production of root mucilage, remaining in root tissues at harvest. Due to the contrasting biochemical composition of the 'well known' C3-plants, the energy crops sorghum and Sudan grass solely differed from pea, oats, and winter wheat, as their crop residues contained huge amounts of water-soluble carbohydrates, in detail 12 - 17 g (100 g DM)$^{-1}$.

On a level with farm fertilisers, digestates, urban compost and char, plant residues constitute one single type of EOC, the one, which is neither microbial nor thermally processed before application, and is indicated as plant-derived C. The processing of plant-derived C came up with a depletion of water-soluble carbohydrates in char (Bruun et al., 2012) and in microbial products (Cotrufo et al., 2013), the enrichment of N in case of microbial processing (Kirkby et al., 2011), the enrichment of C in case of thermal processing, and an increased proportion of organic matter, which was insoluble in sulfuric acid and is commonly referred to as lignin (Table 20). The biochemical composition therefore reflected fundamental biochemical changes during microbial and thermal processing of plant-derived EOC and it remained fragile, if a common biochemical indication of C_{pot} for different types of EOC was feasible.

As agricultural humus balancing is a consecutive process, beginning with the evaluation of the C need of a crop species/ cropping system and ending with the evaluation of C supply by different types of EOC (organic amendments), we supposed to consecutively assess two estimating equations of C_{pot}, as we identified a biochemical indicator for plant residues and then tried to identify a biochemical indicator for all types of EOC, which of course was not intended to reliably differentiate between plant residues of different crop species. According to the highly variable biochemical composition, the EOC-induced CO_2-release and C_{pot} varied, depending on the type of plant residue, the crop species / cropping system

(Table 14, Table 15, Figure 23, and Figure 24), and the type of EOC (Table 21). Beginning with the indicator for plant residues, C_{pot} of litter remained relatively constant, while C_{pot} of stubbles, coarse roots, and fine roots depended on the crop species and the cropping system. The independence of litter decomposition from different crop species was expectable, as mineral nutrients and assimilates were being translocated into other plant organs before senescence, precipitation washed out water-soluble carbohydrates, and fallen litter had been exposed to macro- and microbial access. Litter induced the release of a large proportion of C (about 80 % of initial litter-C) as CO_2 during decomposition in incubation, whereas the decomposition of fine roots resulted in the large amounts of C_{pot}. Similar to litter, fine roots are continuously deposited, but then remain in a canal system ('rhizosheaths'), which they influenced by mucilage deposition during lifetime (Rasse et al., 2005). Neither immediate microbial access nor leaching of carbohydrates might have considerably altered biochemical composition of fine roots before incubation. Fine roots were suspected to the formation of SOC on both pathways, the microbial path, on which water-soluble substances are transferred into mineral-associated SOC and the physical transfer path, on which lignin and low-accessible cell-wall constituents are transferred into coarse particles (as described in Cotrufo et al., 2015). Stubbles and coarse roots, both forming the root stock, induced more CO_2-release than litter. The highest CO_2-release appeared for stubbles and coarse roots of Sorghum and Sudan grass, which equally contained the largest proportion of water-soluble carbohydrates. This prefigured the microbial path of SOC formation to be of minor importance for incubation experiments than the physical transfer path. Further incubation experiments with small-sized litterbags (so called 'litterpads') on plant residues confirmed this prefigure (data not shown in this dissertation). Plant residues induced more CO_2-release than other types of EOC. Continuing with farm fertilisers, urban composts, digestates and char, the highest C_{pot}-values (about 90-100 % of initial EOC) were obtained for urban composts and biochar (Figure 26). Although digestates, urban composts, and farm fertilisers could not be biochemically distinguished, they largely differed in decomposition of some samples. Therefore the characterisation of biochemical quality might still remain insensitive to important biochemical alterations, which assumedly occur during microbial conversion.

We found evidence for C_{pot} to be predictable by biochemical properties of plant residues and EOC in general, as for the most part the variation of C_{pot} could be explained by biochemical composition (Table 16, Table 22). Plant residues constitute one type of EOC. The C/N-ratio was negatively correlated to C_{pot}. The fact that plant residues varied in N concentration but were of relatively constant carbon concentration, while EOC in general varied more in C than in N concentration, made no difference. This supports the universality of previous biochemical indicators, which are based on the C/N-ratio (e.g Nicolardot et al., 2001). The relative proportions of hemicellulose, cellulose and lignin were tightly correlated to C_{pot}, providing further evidence that more recently found indicators, like the indicator suggested by Lashermes et al. (2009) to be more applicable.

Irrespective of predictability of C_{pot}, we observed a negative correlation of the proportion of water-soluble carbohydrates to C_{pot}. The SOC formation from water-soluble carbohydrates on the microbial pathway remained therefore invisible in C_{pot}. As water-soluble carbohydrates are predestined to induce SOC priming (Fontaine et al., 2004, 2007), C_{pot} might have been apparently decreased. The advantages of C_{pot} as a measure for net-effects on SOC therefore arise with the disadvantage of a reversed representation of the SOC formation from dissolved organic matter on the microbial pathway.

6.4 How does N fertilisation, as applied in different time-scales, affect decomposition of plant residues? (Chapter 5)

The microbial pathway of SOC formation emerges importance in recent research on humification, which focuses on the influence of N on plant residue decomposition, e.g. Kirkby et al. (2014), Manzoni et al. (2012b), and Cotrufo et al. (2013). We focused different time-scales of N effects on decomposition as we observed the influence of N as applied as organic and mineral amendments over a period of 30 years (N fertilisation history) or as applied as mineral N addition directly before incubation (short-term N supply) on straw-induced CO_2-release of the 'Berlin soil'. We found straw-induced CO_2-release to depend on N fertilisation over both time-scales (Figure 29, Figure 30). This was in contrast to Nett et al. (2012), neglecting influences of soil fertilisation history on decomposition of plant residues. The IOSDV focused on long-term fertilisation effects, which could be attributed to different N loads or different forms of N fertilisation, as other plant nutrients were supplied according to good manufacturing practice (Winkelmann et al., 2006). Nitrogen fertilisation led to an increased straw-induced CO_2-release in the beginning of the incubation, but to a decreased straw-induced CO_2-release in the end of the incubation. In total, straw-induced CO_2-release was decreased by N fertilisation in both cases. These results confirmed previous incubation experiments by Henriksen and Breland (1999), which had been conducted for shorter incubation durations, but the longer incubation duration enabled a different implication for persistence of straw-C in soil. As N fertilisation increased C_{pot} of straw, implicitly the fraction of straw C to maintain for SOC, persistence of straw-C in soil is equally increased. The effect of long-term fertilisation was lower than the effect of short-term N supply on C_{pot} and in support of Nett et al. (2012) it remained fragile, if they could be statistically identified in an incubation experiment, which contained all EOC types of the IOSDV in a full-factorial design. Omitted amendments in the long-term could easily be compensated by mineral N supplementation in parallel to straw incorporation (supporting N balancing fertilisation).

In concordance with similar investigation, we suggested the N-induced increment of straw-C, remaining in soil and maintaining SOC, to be due to an enhanced CUE of decomposing microorganisms (Kirkby et al., 2013, Kirkby et al., 2014, Cotrufo et al., 2013). We aimed to realise these processes at the 3rd day of incubation, as EOC-induced CO_2-release after this incubation duration still remained an important parameter for C_{pot} prediction equations (Lashermes et al., 2009). In the initial 3 days of incubation we observed an increased microbial growth in soil columns, to which mineral N was supplied before

incubation, whereas an effect of long-term fertilisation could not be verified. As both, straw-induced CO_2-release and microbial growth were increased, an influence of N supply on microbial carbon use efficiency could not be verified in this period. The N supplementation therefore accelerated microbial decomposition of straw but an effect on CUE presumably occurs in subsequent decomposition stages. An effect of mineral N supply on CUE of straw would have been measurable after 56 days of incubation (before both courses of cumulative carbon loss intersected).

After the incubation experiment, we observed mineral N concentration and pH-value in the soil. These parameters provided an informative basis for increased CUE by mineral N supply (Table 31, Table 32). Straw-induced N immobilisation occurred in each of the differently fertilised soils, but it was highest for the ones, which were fertilised with mineral N directly before incubation. As mineral N was immobilised during straw decomposition, it possibly had been metabolised by microorganisms and was converted into microbial products or microbial necromass (Cotrufo et al., 2013). After the incubation experiment, the soil pH was even highest for the samples to which straw and mineral N had been supplied, providing further support for enhanced immobilisation of mineral nitrogenous compounds into organic compounds after mineral N supply. The soil-pH further depended on long-term N fertilisation. The increased immobilisation and pH-values therefore support the framework of CUE, according to which the conversion of plant-derived C into SOC depends on N availability (Cotrufo et al., 2013).

6.5 Conclusion

In conclusion of the first question, the results provided evidence for the reliability of the incubation methodology to assess relative differences in the fraction of EOC, which is transformed into SOC for different types and groups of EOC, as the EOC-induced CO_2-release indirectly determined C_{pot} robustly towards (i) different mathematical models, (ii) different incubation soils, and (iii) subsequent C-supply to soil. Although each of the three settings influenced C_{pot}, the effect was low compared to the differences between C_{pot} of different types of EOC. Nevertheless, C_{pot} was not robust towards a decreased incubation temperature, wherefore incubation experiments should stick to a common standard temperature. Although C_{pot} and EOC-induced CO_2-release both were related to the amount of initially added EOC, they accounted for both EOC mineralisation and SOC priming. C_{pot} represented the net-effect of both and therefore rather accounted for a ‘net-effect on SOC after EOC application’ rather than exclusively the fraction of EOC, which is transferred into SOC. This definition is even closer to the requirements of agricultural humus balancing concepts, which require a coefficient expressing the potential SOC maintenance or ‘humus reproduction’ of a certain type of EOC or plant residue (Andren and Katterer, 1997, Brock et al., 2013, Ebertseder et al., 2014).

In conclusion of the second question, plant residues and further types of EOC largely varied in C_{pot}, which depended on biochemical composition for the most case. The current indicator I_{pot} for EOC by Lashermes et al. (2009) was able to predict C_{pot} for all groups of EOC at good accordance, irrespective of microbial or thermal processing. The complementation of this indicator by further ones, which are

exclusively based on biochemical properties required the integration of more biochemical parameters than simply fractions of cell-wall constituents. For EOC, the integration of carbon concentrations into biochemical indicators covered both easily accessible C, such as the proportion of water-soluble carbohydrates or cellulose, and less accessible C, such as thermally processed C. As both microbial and thermal processing increased the acid-insoluble portion, which is indicated as lignin, one common indicator for all types of EOC was possible: $I_{pot} = 924 – 1.9\ C + 2.0\ LIC$, ($R^2 = 0.92$, $n = 30$). As the variability of biochemical parameters differed in plant residues and other types of EOC, two separate biochemical indicators better applied for prediction of C_{pot} than one universal indicator. In plant residues, the C concentration remained relatively constant, whereas the proportion of nitrogen (N), water-soluble carbohydrates (WSC) and cellulose (CEL) largely varied (Jensen et al., 2005). This is related to the necessity of a separate indicator for C_{pot} of plant residues: $I_{pot} = 269 + 13\ N – 0.5\ WSC + 0.7\ CEL + 1.5\ LIC$ ($R^2 = 0.84$, $n = 40$). However, this indicator did not provide further evidence for the representativeness of C_{pot}, as both pathways of SOC formation were not reflected (as described in Cotrufo et al., 2015). Potential residual organic carbon was rather dedicated to evaluate the potential of certain plant residues for SOC maintenance than to identify basic processes of SOC formation.

In conclusion of the third question, N fertilisation increased C_{pot} of straw. The magnitude of this increase depended on the time-scale of N-fertilisation, whereby direct N-supply increased C_{pot} more than N fertilisation history. The direct application of mineral N was able to compensate for omitted N fertilisation in the long-term. In the initial stage of decomposition, at the 3rd day of incubation, mineral N supply increased both microbial growth and microbial activity, while CUE remained unaffected. As mineral N supply led to increased N immobilisation and increased soil pH during straw decomposition, it may be assumed, that CUE is likewise increased in later decomposition stages. This provides support to previous investigation on microbial CUE under altered N-levels in agricultural ecosystems, namely Manzoni et al. (2012b), Cotrufo et al. (2013), Kirkby et al. (2013), and Kirkby et al. (2014).

In total, C_{pot} robustly represented the fraction of EOC, remaining in soil and maintaining SOC, towards environmental parameter settings, such as soil type and different modes of carbon availability. C_{pot} largely depended on the type of EOC, e. g. plant residues, farm fertilisers, urban composts, digestates, or char. Persistence of different types of EOC therefore substantially influences its applicability in crop cultivation. Potential residual organic carbon of plant residues further depended on the plant residue type and crop species. Different cropping systems therefore require different amendments of EOC to compensate for carbon losses via heterotrophic respiration. Moreover, the fertilisation practice influenced C_{pot} of straw (in general plant residues), emphasizing importance as central part of agricultural management. Persistence of plant residues and EOC therefore remains a cultivation property, which largely depends on the biochemical composition of plant residues and EOC and on agricultural management of a certain site.

6.6 Perspective

Incubation experiments constitute a valuable alternative to long-term field experiments in humus balancing research, as they enable the comparison of several types of EOC under equivalent conditions in a short period of time. For the interpretation of incubation results, further investigation should consider the priming effect as a determining factor of C_{pot}. As the microbial transfer path of dissolved organic matter (Cotrufo et al., 2015) still remained underrepresented by C_{pot}, one should prove if this would be the case, when exclusively EOC mineralisation is regarded. However, C_{pot} remains a highly informative variable, as it is tightly correlated to biochemical properties of EOC. If C_{pot} actually represents the fraction of EOC, remaining and maintaining SOC, should be proven in parallel litterbag experiments, which are exposed to field conditions and laboratory conditions.

Another considerable factor for C_{pot} is the incubation soil. Although the influence of different soils on EOC decomposition is relatively low, the focus on interactions between soil properties and biochemical properties of EOC might open up new perspectives for the interpretation of humification processes in different stages of EOC decomposition. As biochemical properties of EOC were not able to substitute EOC-induced CO_2-release of the initial 3 days of incubation (C_{3d}) in advance for estimating equations of C_{pot} and microbial CUE not yet responded on environmental alteration, e.g. altered N availability within this period of incubation, the detection of soil properties, influencing C_{3d} might help to identify relevant biochemical properties of EOC to improve the biochemical indication of C_{pot}.

In general, future research should focus on EOC-induced carbon fluxes by isotopic tracing to generate knowledge of the quantities of basic processes in an incubation vessel. The parallel measurement of microbial growth from EOC, microbial respiration from EOC, and SOC formation from EOC might not solely enhance the interpretation of integrated parameters such as EOC-induced CO_2-release and C_{pot}, but also enable the comparison of more complex decomposition models such as presented by Manzoni et al. (2012a). The influence of temperature and environmental alterations as typical for agricultural management, e. g. mineral nitrogen supply on SOC formation could be more intensively analysed and interpreted in such densely examined incubation experiments.

Bibliography

1999. ISO 8245. *Water quality - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).* Berlin: Beuth-Wiley-VCH.

2000. DIN 19684. *Methods of soil investigations for agricultural water engineering - Chemical laboratory tests.* Berlin: Beuth-Wiley-VCH.

2005a. DIN 16072. *Soil quality - Laboratory methods for determination of microbial soil respiration.* Berlin: Beuth Wiley-VCH.

2005b. DIN 19746. *Soil quality - Determination of mineral nitrogen (nitrat and ammonium) in soil profiles (Nmin laboratory method).* Berlin: Beuth-Wiley-VCH.

2011. DIN EN ISO 14240-2. *Soil quality - Determination of soil microbial biomass - Part 2: Fumigation-extraction method.* Berlin: Beuth-Wiley-VCH.

2012a. DIN EN 16168. *Sludge, treated biowaste and soil - Determination of total nitrogen using dry combustion method.* Berlin: Beuth Wiley-VCH.

2012b. EN 15936. *Sludge, treated biowaste, soil and waste - Determination of total organic carbon (TOC) by dry combustion.* Berlin: Beuth Wiley-VCH.

ABIVEN, S., HEIM, A. & SCHMIDT, M. W. I. 2011. Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil. *Plant and Soil,* 343**,** 369-378.

ABIVEN, S., RECOUS, S., REYES, V. & OLIVER, R. 2005. Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. *Biology and Fertility of Soils,* 42**,** 119-128.

ABIVEN, S., SCHMIDT, M. W. I. & LEHMANN, J. 2014. Biochar by design. *Nature Geoscience,* 7**,** 326-327.

ALBURQUERQUE, J. A., DE LA FUENTE, C. & BERNAL, M. P. 2012. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agriculture Ecosystems & Environment,* 160**,** 15-22.

AMELUNG, W., BRODOWSKI, S., SANDHAGE-HOFMANN, A. & BOL, R. 2008. COMBINING BIOMARKER WITH STABLE ISOTOPE ANALYSES FOR ASSESSING THE TRANSFORMATION AND TURNOVER OF SOIL ORGANIC MATTER. *In:* SPARKS, D. L. (ed.) *Advances in Agronomy, Vol 100.* San Diego: Elsevier Academic Press Inc.

AMIN, B. A. Z., CHABBERT, B., MOORHEAD, D. & BERTRAND, I. 2014. Impact of fine litter chemistry on lignocellulolytic enzyme efficiency during decomposition of maize leaf and root in soil. *Biogeochemistry,* 117**,** 169-183.

ANDREN, O. & KATTERER, T. 1997. ICBM: The introductory carbon balance model for exploration of soil carbon balances. *Ecological Applications,* 7**,** 1226-1236.

ANEX, R. P., LYND, L. R., LASER, M. S., HEGGENSTALLER, A. H. & LIEBMAN, M. 2007. Potential for enhanced nutrient cycling through coupling of agricultural and bioenergy systems. *Crop Science,* 47**,** 1327-1335.

AULEN, M., SHIPLEY, B. & BRADLEY, R. 2012. Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits. *Annals of Botany,* 109**,** 287-297.

BAATH, E., LUNDGREN, B. & SODERSTROM, B. 1981. EFFECTS OF NITROGEN-FERTILIZATION ON THE ACTIVITY AND BIOMASS OF FUNGI AND BACTERIA IN A PODZOLIC SOIL. *Zentralblatt Fur Bakteriologie Mikrobiologie Und Hygiene I Abteilung Originale C-Allgemeine Angewandte Und Okologische Mikrobiologie,* 2**,** 90-98.

BAKER, J. M. & GRIFFIS, T. J. 2009. Evaluating the potential use of winter cover crops in corn-soybean systems for sustainable co-production of food and fuel. *Agricultural and Forest Meteorology,* 149**,** 2120-2132.

BALDOCK, J. A., OADES, J. M., NELSON, P. N., SKENE, T. M., GOLCHIN, A. & CLARKE, P. 1997. Assessing the extent of decomposition of natural organic materials using solid-state C-13 NMR spectroscopy. *Australian Journal of Soil Research,* 35**,** 1061-1083.

BAMMINGER, C., MARSCHNER, B. & JUSCHKE, E. 2014. An incubation study on the stability and biological effects of pyrogenic and hydrothermal biochar in two soils. *European Journal of Soil Science,* 65**,** 72-82.

BELLAMY, P. H., LOVELAND, P. J., BRADLEY, R. I., LARK, R. M. & KIRK, G. J. D. 2005. Carbon losses from all soils across England and Wales 1978-2003. *Nature,* 437**,** 245-248.

BERNAL, M. P. & KIRCHMANN, H. 1992. CARBON AND NITROGEN MINERALIZATION AND AMMONIA VOLATILIZATION FROM FRESH, AEROBICALLY AND ANAEROBICALLY TREATED PIG MANURE DURING INCUBATION WITH SOIL. *Biology and Fertility of Soils,* 13**,** 135-141.

BERTRAND, I., PREVOT, M. & CHABBERT, B. 2009. Soil decomposition of wheat internodes of different maturity stages: Relative impact of the soluble and structural fractions. *Bioresource Technology,* 100**,** 155-163.

BIRGE, H. E., CONANT, R. T., FOLLETT, R. F., HADDIX, M. L., MORRIS, S. J., SNAPP, S. S., WALLENSTEIN, M. D. & PAUL, E. A. 2015. Soil respiration is not limited by reductions in microbial biomass during long-term soil incubations. *Soil Biology & Biochemistry,* 81**,** 304-310.

BIROUSTE, M., KAZAKOU, E., BLANCHARD, A. & ROUMET, C. 2012. Plant traits and decomposition: are the relationships for roots comparable to those for leaves? *Annals of Botany,* 109**,** 463-472.

BOLINDER, M. A., ANDREN, O., KATTERER, T. & PARENT, L. E. 2008. Soil organic carbon sequestration potential for Canadian agricultural ecoregions calculated using the Introductory Carbon Balance Model. *Canadian Journal of Soil Science,* 88**,** 451-460.

BOLINDER, M. A., JANZEN, H. H., GREGORICH, E. G., ANGERS, D. A. & VANDENBYGAART, A. J. 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. *Agriculture Ecosystems & Environment,* 118**,** 29-42.

BONANOMI, G., INCERTI, G., GIANNINO, F., MINGO, A., LANZOTTI, V. & MAZZOLENI, S. 2013. Litter quality assessed by solid state C-13 NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios. *Soil Biology & Biochemistry,* 56**,** 40-48.

BORGEN, S. K., MOLSTAD, L., BRUUN, S., BRELAND, T. A., BAKKEN, L. R. & BLEKEN, M. A. 2011. Estimation of plant litter pools and decomposition-related parameters in a mechanistic model. *Plant and Soil,* 338**,** 205-222.

BROADBENT, F. E. 1965. Effect of fertilizer nitrogen on the release of soil nitrogen. *Soil Sci Soc Amer Proc,* 29**,** 692-696.

BROCK, C., FRANKO, U., OBERHOLZER, H.-R., KUKA, K., LEITHOLD, G., KOLBE, H. & REINHOLD, J. 2013. Humus balancing in Central Europe-concepts, state of the art, and further challenges. *Journal of Plant Nutrition and Soil Science,* 176**,** 3-11.

BROMAND, S., WHALEN, J. K., JANZEN, H. H., SCHJOERRING, J. K. & ELLERT, B. H. 2001. A pulse-labelling method to generate C-13- enriched plant materials. *Plant and Soil,* 235**,** 253-257.

BRUUN, E. W., AMBUS, P., EGSGAARD, H. & HAUGGAARD-NIELSEN, H. 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. *Soil Biology & Biochemistry,* 46**,** 73-79.

BRUUN, S., STENBERG, B., BRELAND, T. A., GUDMUNDSSON, J., HENRIKSEN, T. M., JENSEN, L. S., KORSAETH, A., LUXHOI, J., PALMASON, F., PEDERSEN, A. & SALO, T. 2005. Empirical predictions of plant material C and N mineralization patterns from near infrared spectroscopy, stepwise chemical digestion and C/N ratios. *Soil Biology & Biochemistry,* 37**,** 2283-2296.

BUSTAMANTE, M. A., ALBURQUERQUE, J. A., RESTREPO, A. P., DE LA FUENTE, C., PAREDES, C., MORAL, R. & BERNAL, M. P. 2012. Co-composting of the solid fraction of anaerobic digestates, to obtain added-value materials for use in agriculture. *Biomass & Bioenergy,* 43**,** 26-35.

CABRERA, M. L. & BEARE, M. H. 1993. ALKALINE PERSULFATE OXIDATION FOR DETERMINING TOTAL NITROGEN IN MICROBIAL BIOMASS EXTRACTS. *Soil Science Society of America Journal,* 57**,** 1007-1012.

CAMPBELL, N. A., REECE, J. B. & MITCHELL, L. G. 1999. *Biology. Fifth edition*, Addison-Wesley.

CARPENTER-BOGGS, L., KENNEDY, A. C. & REGANOLD, J. P. 2000. Organic and biodynamic management: Effects on soil biology. *Soil Science Society of America Journal,* 64**,** 1651-1659.

CARVALHAIS, N., FORKEL, M., KHOMIK, M., BELLARBY, J., JUNG, M., MIGLIAVACCA, M., MU, M. Q., SAATCHI, S., SANTORO, M., THURNER, M., WEBER, U., AHRENS, B., BEER, C., CESCATTI, A., RANDERSON, J. T. & REICHSTEIN, M. 2014. Global covariation of carbon turnover times with climate in terrestrial ecosystems. *Nature,* 514**,** 213-+.

CHAPIN, F. S., WOODWELL, G. M., RANDERSON, J. T., RASTETTER, E. B., LOVETT, G. M., BALDOCCHI, D. D., CLARK, D. A., HARMON, M. E., SCHIMEL, D. S., VALENTINI, R., WIRTH, C., ABER, J. D., COLE, J. J., GOULDEN, M. L., HARDEN, J. W., HEIMANN, M., HOWARTH, R. W., MATSON, P. A., MCGUIRE, A. D., MELILLO, J. M., MOONEY, H. A., NEFF, J. C., HOUGHTON, R. A., PACE, M. L., RYAN, M. G., RUNNING, S. W., SALA, O. E., SCHLESINGER, W. H. & SCHULZE, E. D. 2006. Reconciling carbon-cycle concepts, terminology, and methods. *Ecosystems,* 9**,** 1041-1050.

CHEN, S. T., HUANG, Y., ZOU, J. W. & SHI, Y. S. 2013. Mean residence time of global topsoil organic carbon depends on temperature, precipitation and soil nitrogen. *Global and Planetary Change,* 100**,** 99-108.

COBAN, H., MILTNER, A., ELLING, F. J., HINRICHS, K. U. & KASTNER, M. 2015. The contribution of biogas residues to soil organic matter formation and CO2 emissions in an arable soil. *Soil Biology & Biochemistry,* 86**,** 108-115.

CONANT, R. T., RYAN, M. G., AGREN, G. I., BIRGE, H. E., DAVIDSON, E. A., ELIASSON, P. E., EVANS, S. E., FREY, S. D., GIARDINA, C. P., HOPKINS, F. M., HYVONEN, R., KIRSCHBAUM, M. U. F., LAVALLEE, J. M., LEIFELD, J., PARTON, W. J., STEINWEG, J. M., WALLENSTEIN, M. D., WETTERSTEDT, J. A. M. & BRADFORD, M. A. 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. *Global Change Biology,* 17**,** 3392-3404.

CONG, W. F., HOFFLAND, E., LI, L., JANSSEN, B. H. & VAN DER WERF, W. 2015. Intercropping affects the rate of decomposition of soil organic matter and root litter. *Plant and Soil,* 391**,** 399-411.

COTRUFO, M. F., SOONG, J. L., HORTON, A. J., CAMPBELL, E. E., HADDIX, M. L., WALL, D. H. & PARTON, A. J. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. *Nature Geoscience,* 8**,** 776-+.

COTRUFO, M. F., WALLENSTEIN, M. D., BOOT, C. M., DENEF, K. & PAUL, E. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global Change Biology,* 19**,** 988-995.

CRAINE, J. M., FIERER, N. & MCLAUCHLAN, K. K. 2010. Widespread coupling between the rate and temperature sensitivity of organic matter decay. *Nature Geoscience,* 3**,** 854-857.

CRAINE, J. M., MORROW, C. & FIERER, N. 2007. Microbial nitrogen limitation increases decomposition. *Ecology,* 88**,** 2105-2113.

CREAMER, C. A., FILLEY, T. R., BOUTTON, T. W., OLEYNIK, S. & KANTOLA, I. B. 2011. Controls on soil carbon accumulation during woody plant encroachment: Evidence from physical fractionation, soil respiration, and δ13C of respired CO2. *Soil Biology and Biochemistry,* 43**,** 1678-1687.

DAMBREVILLE, C., HALLET, S., NGUYEN, C., MORVAN, T., GERMON, J. C. & PHILIPPOT, L. 2006. Structure and activity of the denitrifying community in a maize-cropped field fertilized with composted pig manure or ammonium nitrate. *Fems Microbiology Ecology,* 56**,** 119-131.

DAVIDSON, E. A. & JANSSENS, I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature,* 440**,** 165-173.

DE GRAAFF, M.-A., SIX, J., JASTROW, J. D., SCHADT, C. W. & WULLSCHLEGER, S. D. 2013. Variation in root architecture among switchgrass cultivars impacts root decomposition rates. *Soil Biology & Biochemistry,* 58**,** 198-206.

DE NEVE, S., SLEUTEL, S. & HOFMAN, G. 2003. Carbon mineralization from composts and food industry wastes added to soil. *Nutrient Cycling in Agroecosystems,* 67**,** 13-20.

DENEVE, S., PANNIER, J. & HOFMAN, G. 1996. Temperature effects on C- and N-mineralization from vegetable crop residues. *Plant and Soil,* 181**,** 25-30.

DICK, R. P., RASMUSSEN, P. E. & KERLE, E. A. 1988. INFLUENCE OF LONG-TERM RESIDUE MANAGEMENT ON SOIL ENZYME-ACTIVITIES IN RELATION TO SOIL CHEMICAL-PROPERTIES OF A WHEAT-FALLOW SYSTEM. *Biology and Fertility of Soils,* 6**,** 159-164.

DON, A., RODENBECK, C. & GLEIXNER, G. 2013. Unexpected control of soil carbon turnover by soil carbon concentration. *Environmental Chemistry Letters,* 11**,** 407-413.

DUMAS, J. B. A. 1831. Procedes de l'analyse organique. *Ann. Chim. Phys.,* 2**,** 198-2013.

DUNGAIT, J. A. J., HOPKINS, D. W., GREGORY, A. S. & WHITMORE, A. P. 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology,* 18**,** 1781-1796.

DWD. 2015. *Climate data for Germany: Long-term means* [Online]. Federal Ministry of transport and digital infrastracture. Available: http://www.dwd.de [Accessed 2015].

EBERTSEDER, T., ENGELS, C., HEYN, J., REINHOLD, J., BROCK, C., FÜRSTENFELD, F., HÜLSBERGEN, K. J., ISERMANN, K., KOLBE, H., LEITHOLD, G., SCHMID, H., SCHWEITZER, K., WILLMS, M. & ZIMMER, J. 2014. Humusbilanzierung - Methode zur Analyse und Bewertung der Humusversorgung von Ackerland. *Standpunkt des VDLUFA.* Speyer: Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA).

EKSCHMITT, K., LIU, M. Q., VETTER, S., FOX, O. & WOLTERS, V. 2005. Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil? *Geoderma,* 128**,** 167-176.

ENGELS, C. 2011. Bedeutung des Pflanzenbedingten Eintrages von organischem Kohlenstoff in den Boden für die Humusbilanzierung. VDLUFA-Kongress, 2011 Speyer. Speyer.

ENGELS, C., KIRKBY, E. & WHITE, P. 2012. *Mineral Nutrition, Yield and Source-Sink Relationships*.

ERNST, G., MUELLER, A., GOEHLER, H. & EMMERLING, C. 2008. C and N turnover of fermented residues from biogas plants in soil in the presence of three different earthworm species (Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa). *Soil Biology & Biochemistry,* 40**,** 1413-1420.

FAUCI, M. F. & DICK, R. P. 1994. SOIL MICROBIAL DYNAMICS - SHORT-TERM AND LONG-TERM EFFECTS OF INORGANIC AND ORGANIC NITROGEN. *Soil Science Society of America Journal,* 58**,** 801-806.

FINN, D., PAGE, K., CATTON, K., STROUNINA, E., KIENZLE, M., ROBERTSON, F., ARMSTRONG, R. & DALAL, R. 2015. Effect of added nitrogen on plant litter decomposition depends on initial soil carbon and nitrogen stoichiometry. *Soil Biology & Biochemistry,* 91**,** 160-168.

FISCHLIN, A., MIDGLEY, G. F., PRICE, J. T. L., R., GOPAL, B., TURLEY, C., ROUNSEVELL, M. D. A., DUBE, O. P., TARAZONA, J. & VELICHKO, A. A. 2007. Ecosystems, their properties, goods, and services *In:* PARRY, M. L., CANZIANI, O. F., PALUTIKOF, J. P., VAN DER LINDEN, P. J. & HANSON, C. E. (eds.) *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, UK and New York, NY, USA: Cambridge University Press.

FISHER, S. G. & LIKENS, G. E. 1973. ENERGY FLOW IN BEAR BROOK, NEW HAMPSHIRE - INTEGRATIVE APPROACH TO STREAM ECOSYSTEM METABOLISM. *Ecological Monographs,* 43**,** 421-439.

FOG, K. 1988. THE EFFECT OF ADDED NITROGEN ON THE RATE OF DECOMPOSITION OF ORGANIC-MATTER. *Biological Reviews of the Cambridge Philosophical Society,* 63**,** 433-462.

FONTAINE, S., BARDOUX, G., ABBADIE, L. & MARIOTTI, A. 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters,* 7**,** 314-320.

FONTAINE, S. & BAROT, S. 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecology Letters,* 8**,** 1075-1087.

FONTAINE, S., BAROT, S., BARRE, P., BDIOUI, N., MARY, B. & RUMPEL, C. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature,* 450**,** 277-U10.

FRANKO, U. 1997. Modellierung des umsatzes der organischen bodensubstanz. *Archives of Agronomy and Soil Science,* 41**,** 527-547.

FRANKO, U. & OELSCHLÄGEL, B. 1995. Einfluss von Klima und Textur auf die biologische Aktivität beim Umsatz der organischen Bodensubstanz. *Archiv Acker- Pflanzenbau Bodenkunde,* 39**,** 155-163.

GARTNER, T. B. & CARDON, Z. G. 2004. Decomposition dynamics in mixed-species leaf litter. *Oikos,* 104**,** 230-246.

GOFF, B. M., MOORE, K. J., FALES, S. L. & HEATON, E. A. 2010. Double-Cropping Sorghum for Biomass. *Agronomy Journal,* 102**,** 1586-1592.

GRABBER, J. H. & COBLENTZ, W. K. 2009. Polyphenol, Conditioning, and Conservation Effects on Protein Fractions and Degradability in Forage Legumes. *Crop Science,* 49**,** 1511-1522.

GRIGATTI, M., DI GIROLAMO, G., CHINCARINI, R., CIAVATTA, C. & BARBANTI, L. 2011. Potential nitrogen mineralization, plant utilization efficiency and soil CO2 emissions following the addition of anaerobic digested slurries. *Biomass & Bioenergy,* 35**,** 4619-4629.

GUNAPALA, N. & SCOW, K. M. 1998. Dynamics of soil microbial biomass and activity in conventional and organic farming systems. *Soil Biology & Biochemistry,* 30**,** 805-816.

HADAS, A., KAUTSKY, L. & PORTNOY, R. 1996. Mineralization of composted manure and microbial dynamics in soil as affected by long-term nitrogen management. *Soil Biology & Biochemistry,* 28**,** 733-738.

HAMMES, K., TORN, M. S., LAPENAS, A. G. & SCHMIDT, M. W. I. 2008. Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences,* 5**,** 1339-1350.

HARVARD. 2015. *Global position finder* [Online]. Center for geographic analysis, Harvard University. Available: http://maps.cga.harvard.edu/gpf/ [Accessed 2015].

HATFIELD, R. & FUKUSHIMA, R. S. 2005. Can lignin be accurately measured? *Crop Science,* 45**,** 832-839.

HÉNIN, S. & DUPUIS, M. 1945. A tentative balance sheet for soil organic matter.

Essai de bilan de la matière organique des sols. *Annales Agronomiques,* 15**,** 17-29.

HENRIKSEN, T. M. & BRELAND, T. A. 1999. Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. *Soil Biology & Biochemistry,* 31**,** 1121-1134.

HENRIKSEN, T. M., KORSAETH, A., BRELAND, T. A., STENBERG, B., JENSEN, L. S., BRUUN, S., GUDMUNDSSON, J., PALMASON, F., PEDERSEN, A. & SALO, T. J. 2007. Stepwise chemical digestion, near-infrared spectroscopy or total N measurement to take account of decomposability of plant C and N. *Soil Biology & Biochemistry,* 39**,** 3115-3126.

HÖCKER, S., BESSLER, H., MEWES, P. & ENGELS, C. 25.06.2015 2015. *RE: Plant-derived Carbon input in an energy crop cultivation field experiment in Berlin Dahlem (2012-2014).*

HOUOT, S. & CHAUSSOD, R. 1995. IMPACT OF AGRICULTURAL PRACTICES ON THE SIZE AND ACTIVITY OF THE MICROBIAL BIOMASS IN A LONG-TERM FIELD EXPERIMENT. *Biology and Fertility of Soils,* 19**,** 309-316.

JANSSENS, I. A. & PILEGAARD, K. 2003. Large seasonal changes in Q(10) of soil respiration in a beech forest. *Global Change Biology,* 9**,** 911-918.

JENSEN, H. L. 1929. On the influence of the carbon: Nitrogen ratios of organic material on the mineralisation of nitrogen. *Journal of Agricultural Science,* 19**,** 71-82.

JENSEN, L. S., SALO, T., PALMASON, F., BRELAND, T. A., HENRIKSEN, T. M., STENBERG, B., PEDERSEN, A., LUNDSTROM, C. & ESALA, M. 2005. Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. *Plant and Soil,* 273**,** 307-326.

JOERGENSEN, R. G. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EC) value. *Soil Biology & Biochemistry,* 28**,** 25-31.

JOERGENSEN, R. G. & MUELLER, T. 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EN) value. *Soil Biology & Biochemistry,* 28**,** 33-37.

JONES, D. L., NGUYEN, C. & FINLAY, R. D. 2009. Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant and Soil,* 321**,** 5-33.

KANDELER, E., STEMMER, M. & KLIMANEK, E. M. 1999. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. *Soil Biology & Biochemistry,* 31**,** 261-273.

KARHU, K., AUFFRET, M. D., DUNGAIT, J. A. J., HOPKINS, D. W., PROSSER, J. I., SINGH, B. K., SUBKE, J. A., WOOKEY, P. A., AGREN, G. I., SEBASTIA, M. T., GOURIVEAU, F., BERGKVIST, G., MEIR, P., NOTTINGHAM, A. T., SALINAS, N. & HARTLEY, I. P. 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature,* 513**,** 81-+.

KARPENSTEIN-MACHAN, M. 2001. Sustainable cultivation concepts for domestic energy production from biomass. *Critical Reviews in Plant Sciences,* 20**,** 1-14.

KATTERER, T., BORJESSON, G. & KIRCHMANN, H. 2014. Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. *Agriculture Ecosystems & Environment,* 189**,** 110-118.

KAUTZ, T. 2005. *Bodenökologische Wirkungen differenzierter organisch-mineralischer Düngung an verschiedenen Standorten*, Cuvillier.

KAYLER, Z. E., KAISER, M., GESSLER, A., ELLERBROCK, R. H. & SOMMER, M. 2011. Application of delta C-13 and delta N-15 isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms. *Biogeosciences,* 8**,** 2895-2906.

KEILUWEIT, M., NICO, P. S., JOHNSON, M. G. & KLEBER, M. 2010. Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). *Environmental Science & Technology,* 44**,** 1247-1253.

KEMMITT, S. J., LANYON, C. V., WAITE, I. S., WEN, Q., ADDISCOTT, T. M., BIRD, N. R. A., O'DONNELL, A. G. & BROOKES, P. C. 2008. Mineralization of native soil organic matter is not regulated by the size, activity or composition of the soil microbial biomass - a new perspective. *Soil Biology & Biochemistry,* 40**,** 61-73.

KIEM, R. & KOGEL-KNABNER, I. 2002. Refractory organic carbon in particle-size fractions of arable soils II: organic carbon in relation to mineral surface area and iron oxides in fractions < 6 mu m. *Organic Geochemistry,* 33**,** 1699-1713.

KIRKBY, C. A., KIRKEGAARD, J. A., RICHARDSON, A. E., WADE, L. J., BLANCHARD, C. & BATTEN, G. 2011. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. *Geoderma,* 163**,** 197-208.

KIRKBY, C. A., RICHARDSON, A. E., WADE, L. J., BATTENB, G. D., BLANCHARD, C. & KIRKEGAARD, J. A. 2013. Carbon-nutrient stoichiometry to increase soil carbon sequestration. *Soil Biology & Biochemistry,* 60**,** 77-86.

KIRKBY, C. A., RICHARDSON, A. E., WADE, L. J., PASSIOURA, J. B., BATTEN, G. D., BLANCHARD, C. & KIRKEGAARD, J. A. 2014. Nutrient availability limits carbon sequestration in arable soils. *Soil Biology & Biochemistry,* 68**,** 402-409.

KOGEL-KNABNER, I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology & Biochemistry,* 34**,** 139-162.

KÖHN, M. 1928. *Beträge zur Theorie und Praxis der mechanischen Bodenuntersuchung,* Dessau, Gutenberg.

KOLAR, L., KUZEL, S., PETERKA, J., STINDL, P. & PLAT, V. 2008. Agrochemical value of organic matter of fermenter wastes in biogas production. *Plant Soil and Environment,* 54**,** 321-328.

KOU, L., CHEN, W. W., GAO, W. L., YANG, H., WANG, H. M. & LI, S. G. 2015. Effects of mixture of branch order-based roots and nitrogen addition on root decay in a subtropical pine plantation. *Biology and Fertility of Soils,* 51**,** 947-957.

KUZYAKOV, Y. & DOMANSKI, G. 2000. Carbon input by plants into the soil. Review. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* 163**,** 421-431.

KUZYAKOV, Y., FRIEDEL, J. K. & STAHR, K. 2000. Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry,* 32**,** 1485-1498.

LACKNER, K. S. 2003. A guide to CO2 sequestration. *Science,* 300**,** 1677-1678.

LANGMEIER, M., FROSSARD, E., KREUZER, M., MADER, P., DUBOIS, D. & OBERSON, A. 2002. Nitrogen fertilizer value of cattle manure applied on soils originating from organic and conventional farming systems. *Agronomie,* 22**,** 789-800.

LASHERMES, G., NICOLARDOT, B., PARNAUDEAU, V., THURIES, L., CHAUSSOD, R., GUILLOTIN, M. L., LINERES, M., MARY, B., METZGER, L., MORVAN, T., TRICAUD,

A., VILLETTE, C. & HOUOT, S. 2009. Indicator of potential residual carbon in soils after exogenous organic matter application. *European Journal of Soil Science,* 60**,** 297-310.

LEHMANN, J. 2007. A handful of carbon. *Nature,* 447**,** 143-144.

LIBRA, J. A., RO, K. S., KAMMANN, C., FUNKE, A., BERGE, N. D., NEUBAUER, Y., TITIRICI, M.-M., FÜHNER, C., BENS, O., KERN, J. & EMMERICH, K.-H. 2011. Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels,* 2**,** 71-106.

LINDEDAM, J., MAGID, J., POULSEN, P. & LUXHOI, J. 2009. Tissue architecture and soil fertility controls on decomposer communities and decomposition of roots. *Soil Biology & Biochemistry,* 41**,** 1040-1049.

LIU, H. S., LI, L. H., HAN, X. G., HUANG, J. H., SUN, J. X. & WANG, H. Y. 2006. Respiratory substrate availability plays a crucial role in the response of soil respiration to environmental factors. *Applied Soil Ecology,* 32**,** 284-292.

LIU, L. L. & GREAVER, T. L. 2010. A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters,* 13**,** 819-828.

LIU, P., SUN, O. J., HUANG, J., LI, L. & HAN, X. 2007. Nonadditive effects of litter mixtures on decomposition and correlation with initial litter N and P concentrations in grassland plant species of northern China. *Biology and Fertility of Soils,* 44**,** 211-216.

LOVETT, G., COLE, J. & PACE, M. 2006. Is net ecosystem production equal to ecosystem carbon accumulation? *Ecosystems,* 9**,** 152-155.

LU, F., LI, T. S., WANG, T. F., SHAO, L. M. & HE, P. J. 2014. Improvement of sludge digestate biodegradability by thermophilic bioaugmentation. *Applied Microbiology and Biotechnology,* 98**,** 969-977.

LUYSSAERT, S., INGLIMA, I., JUNG, M., RICHARDSON, A. D., REICHSTEIN, M., PAPALE, D., PIAO, S. L., SCHULZES, E. D., WINGATE, L., MATTEUCCI, G., ARAGAO, L., AUBINET, M., BEERS, C., BERNHOFER, C., BLACK, K. G., BONAL, D., BONNEFOND, J. M., CHAMBERS, J., CIAIS, P., COOK, B., DAVIS, K. J., DOLMAN, A. J., GIELEN, B., GOULDEN, M., GRACE, J., GRANIER, A., GRELLE, A., GRIFFIS, T., GRUNWALD, T., GUIDOLOTTI, G., HANSON, P. J., HARDING, R., HOLLINGER, D. Y., HUTYRA, L. R., KOLAR, P., KRUIJT, B., KUTSCH, W., LAGERGREN, F., LAURILA, T., LAW, B. E., LE MAIRE, G., LINDROTH, A., LOUSTAU, D., MALHI, Y., MATEUS, J., MIGLIAVACCA, M., MISSON, L., MONTAGNANI, L., MONCRIEFF, J., MOORS, E., MUNGER, J. W., NIKINMAA, E., OLLINGER, S. V., PITA, G., REBMANN, C., ROUPSARD, O., SAIGUSA, N., SANZ, M. J., SEUFERT, G., SIERRA, C., SMITH, M. L., TANG, J., VALENTINI, R., VESALA, T. & JANSSENS, I. A. 2007. CO2 balance of boreal, temperate, and tropical forests derived from a global database. *Global Change Biology,* 13**,** 2509-2537.

LUYSSAERT, S., SCHULZE, E. D., BORNER, A., KNOHL, A., HESSENMOLLER, D., LAW, B. E., CIAIS, P. & GRACE, J. 2008. Old-growth forests as global carbon sinks. *Nature,* 455**,** 213-215.

MACHINET, G. E., BERTRAND, I., BARRIERE, Y., CHABBERT, B. & RECOUS, S. 2011. Impact of plant cell wall network on biodegradation in soil: Role of lignin composition and phenolic acids in roots from 16 maize genotypes. *Soil Biology & Biochemistry,* 43**,** 1544-1552.

MAESTRINI, B., NANNIPIERI, P. & ABIVEN, S. 2015. A meta-analysis on pyrogenic organic matter induced priming effect. *Global Change Biology Bioenergy,* 7**,** 577-590.

MALGHANI, S., GLEIXNER, G. & TRUMBORE, S. E. 2013. Chars produced by slow pyrolysis and hydrothermal carbonization vary in carbon sequestration potential and greenhouse gases emissions. *Soil Biology & Biochemistry,* 62**,** 137-146.

MANATT, R. K., HALLAM, A., SCHULTE, L. A., HEATON, E. A., GUNTHER, T., HALL, R. B. & MOORE, K. J. 2013. Farm-scale costs and returns for second generation bioenergy cropping systems in the US Corn Belt. *Environmental Research Letters,* 8.

MANZONI, S., PINEIRO, G., JACKSON, R. B., JOBBAGY, E. G., KIM, J. H. & PORPORATO, A. 2012a. Analytical models of soil and litter decomposition: Solutions for mass loss and time-dependent decay rates. *Soil Biology & Biochemistry,* 50**,** 66-76.

MANZONI, S., TAYLOR, P., RICHTER, A., PORPORATO, A. & AGREN, G. I. 2012b. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist,* 196**,** 79-91.

MANZONI, S., TROFYMOW, J. A., JACKSON, R. B. & PORPORATO, A. 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecological Monographs,* 80**,** 89-106.

MARSCHNER, B., BRODOWSKI, S., DREVES, A., GLEIXNER, G., GUDE, A., GROOTES, P. M., HAMER, U., HEIM, A., JANDL, G., JI, R., KAISER, K., KALBITZ, K., KRAMER, C., LEINWEBER, P., RETHEMEYER, J., SCHAEFFER, A., SCHMIDT, M. W. I., SCHWARK, L. & WIESENBERG, G. L. B. 2008. How relevant is recalcitrance for the stabilization of organic matter in soils? *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde,* 171**,** 91-110.

MATHERS, N. J., JALOTA, R. K., DALAL, R. C. & BOYD, S. E. 2007. C-13-NMR analysis of decomposing litter and fine roots in the semi-arid Mulga Lands of southern Queensland. *Soil Biology & Biochemistry,* 39**,** 993-1006.

MAZZILLI, S. R., KEMANIAN, A. R., ERNST, O. R., JACKSON, R. B. & PINEIRO, G. 2014. Priming of soil organic carbon decomposition induced by corn compared to soybean crops. *Soil Biology & Biochemistry,* 75**,** 273-281.

MOORHEAD, D., LASHERMES, G., RECOUS, S. & BERTRAND, I. 2014. Interacting Microbe and Litter Quality Controls on Litter Decomposition: A Modeling Analysis. *Plos One,* 9.

MOORHEAD, K. K., GRAETZ, D. A. & REDDY, K. R. 1987. DECOMPOSITION OF FRESH AND ANAEROBICALLY DIGESTED PLANT BIOMASS IN SOIL. *Journal of Environmental Quality,* 16**,** 25-28.

MORVAN, T., NICOLARDOT, B. & PEAN, L. 2006. Biochemical composition and kinetics of C and N mineralization of animal wastes: a typological approach. *Biology and Fertility of Soils,* 42**,** 513-522.

MUMME, J., ECKERVOGT, L., PIELERT, J., DIAKITE, M., RUPP, F. & KERN, J. 2011. Hydrothermal carbonization of anaerobically digested maize silage. *Bioresource Technology,* 102**,** 9255-9260.

NETT, L., RUPPEL, S., RUEHLMANN, J., GEORGE, E. & FINK, M. 2012. Influence of Soil Amendment History on Decomposition of Recently Applied Organic Amendments. *Soil Science Society of America Journal,* 76**,** 1290-1300.

NICOLARDOT, B., RECOUS, S. & MARY, B. 2001. Simulation of C and N mineralisation during crop residue decomposition: A simple dynamic model based on the C : N ratio of the residues. *Plant and Soil,* 228**,** 83-103.

OLK, D. C. & GREGORICH, E. G. 2006. Overview of the symposium proceedings, "Meaningful pools in determining soil carbon and nitrogen dynamics". *Soil Science Society of America Journal,* 70**,** 967-974.

OTTOW, J. C. G. 2011. *Mikrobiologie von Böden: Biodiversität, Ökophysiologie und Metagenomik*, Springer Berlin Heidelberg.

PAL, D., BROADBENT, F. E. & MIKKELSEN, D. S. 1975. INFLUENCE OF TEMPERATURE ON KINETICS OF RICE STRAW DECOMPOSITION IN SOILS. *Soil Science,* 120**,** 442-449.

PATERSON, E. & SIM, A. 2013. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. *Global Change Biology,* 19**,** 1562-1571.

PELTRE, C., BRUUN, S., DU, C., THOMSEN, I. K. & JENSEN, L. S. 2014. Assessing soil constituents and labile soil organic carbon by mid-infrared photoacoustic spectroscopy. *Soil Biology & Biochemistry,* 77**,** 41-50.

POEPLAU, C., KATTERER, T., BOLINDER, M. A., BORJESSON, G., BERTI, A. & LUGATO, E. 2015. Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments. *Geoderma,* 237**,** 246-255.

RASSE, D. P., RUMPEL, C. & DIGNAC, M. F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil,* 269**,** 341-356.

RÄTZ, D., HAJKOVA, Z. & PAUL, N. 2011. 25 Bioenergieregionen im Porträt. Berlin: Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz.

REDIN, M., RECOUS, S., AITA, C., DIETRICH, G., SKOLAUDE, A. C., LUDKE, W. H., SCHMATZ, R. & GIACOMINI, S. J. 2014. How the chemical composition and heterogeneity

of crop residue mixtures decomposing at the soil surface affects C and N mineralization. *Soil Biology & Biochemistry,* 78**,** 65-75.

RICE, C. W. 2006. Introduction to special section on greenhouse gases and carbon sequestration in agriculture and forestry. *Journal of Environmental Quality,* 35**,** 1338-1340.

ROBIN, D. 1997. Usefulness of organic profiles for evaluating the stable organic matter fraction produced during decomposition in soil and the classification of organic manures. *Agronomie,* 17**,** 157-171.

RUFFO, M. L. & BOLLERO, G. A. 2003. Residue decomposition and prediction of carbon and nitrogen release rates based on biochemical fractions using principal-component regression. *Agronomy Journal,* 95**,** 1034-1040.

RUPPEL, S., TORSVIK, V., DAAE, F. L., OVREAS, L. & RUEHLMANN, J. 2007. Nitrogen availability decreases prokaryotic diversity in sandy soils. *Biology and Fertility of Soils,* 43**,** 449-459.

SALINASGARCIA, J. R., HONS, F. M., MATOCHA, J. E. & ZUBERER, D. A. 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. *Biology and Fertility of Soils,* 25**,** 182-188.

SANGER, A., GEISSELER, D. & LUDWIG, B. 2011. Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry. *Biology and Fertility of Soils,* 47**,** 249-259.

SCHEFFER, F., SCHACHTSCHABEL, P., BLUME, H. P., HARTGE, K. H. & SCHWERTMANN, U. 1979. *TEXT BOOK OF SOIL SCIENCE 10TH EDITION*.

SCHIMEL, D. S., HOUSE, J. I., HIBBARD, K. A., BOUSQUET, P., CIAIS, P., PEYLIN, P., BRASWELL, B. H., APPS, M. J., BAKER, D., BONDEAU, A., CANADELL, J., CHURKINA, G., CRAMER, W., DENNING, A. S., FIELD, C. B., FRIEDLINGSTEIN, P., GOODALE, C., HEIMANN, M., HOUGHTON, R. A., MELILLO, J. M., MOORE, B., MURDIYARSO, D., NOBLE, I., PACALA, S. W., PRENTICE, I. C., RAUPACH, M. R., RAYNER, P. J., SCHOLES, R. J., STEFFEN, W. L. & WIRTH, C. 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature,* 414**,** 169-172.

SCHITTENHELM, S. 2010. Effect of Drought Stress on Yield and Quality of Maize/Sunflower and Maize/Sorghum Intercrops for Biogas Production. *Journal of Agronomy and Crop Science,* 196**,** 253-261.

SCHLESINGER, W. H. & BERNHARDT, E. S. 2013. Chapter 5 - The Biosphere: The Carbon Cycle of Terrestrial Ecosystems. *Biogeochemistry (Third Edition).* Boston: Academic Press.

SCHMIDT, M. W. I. & NOACK, A. G. 2000. Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Biogeochemical Cycles,* 14**,** 777-793.

SCHMIDT, M. W. I., TORN, M. S., ABIVEN, S., DITTMAR, T., GUGGENBERGER, G., JANSSENS, I. A., KLEBER, M., KOGEL-KNABNER, I., LEHMANN, J., MANNING, D. A.

C., NANNIPIERI, P., RASSE, D. P., WEINER, S. & TRUMBORE, S. E. 2011. Persistence of soil organic matter as an ecosystem property. *Nature,* 478**,** 49-56.

SHAHZAD, T., CHENU, C., GENET, P., BAROT, S., PERVEEN, N., MOUGIN, C. & FONTAINE, S. 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. *Soil Biology & Biochemistry,* 80**,** 146-155.

SIX, J., CONANT, R. T., PAUL, E. A. & PAUSTIAN, K. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil,* 241**,** 155-176.

SIX, J., ELLIOTT, E. T. & PAUSTIAN, K. 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology & Biochemistry,* 32**,** 2099-2103.

SLEUTEL, S., DE NEVE, S., ROIBAS, M. R. P. & HOFMAN, G. 2005. The influence of model type and incubation time on the estimation of stable organic carbon in organic materials. *European Journal of Soil Science,* 56**,** 505-514.

SMITH, P., SMITH, J. U., POWLSON, D. S., MCGILL, W. B., ARAH, J. R. M., CHERTOV, O. G., COLEMAN, K., FRANKO, U., FROLKING, S., JENKINSON, D. S., JENSEN, L. S., KELLY, R. H., KLEIN-GUNNEWIEK, H., KOMAROV, A. S., LI, C., MOLINA, J. A. E., MUELLER, T., PARTON, W. J., THORNLEY, J. H. M. & WHITMORE, A. P. 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. *Geoderma,* 81**,** 153-225.

SODERSTROM, B., BAATH, E. & LUNDGREN, B. 1983. DECREASE IN SOIL MICROBIAL ACTIVITY AND BIOMASSES OWING TO NITROGEN AMENDMENTS. *Canadian Journal of Microbiology,* 29**,** 1500-1506.

SPIEGEL, H., DERSCH, G., BAUMGARTEN, A. & HOESCH, J. 2010. The International Organic Nitrogen Long-term Fertilisation Experiment (IOSDV) at Vienna after 21 years. *Archives of Agronomy and Soil Science,* 56**,** 405-420.

SSSA 2008. *Glossary of Soil Science Terms,* Madison, WI, USA, Soil Science Society of America.

STARK, C. H., CONDRON, L. M., O'CALLAGHAN, M., STEWART, A. & DI, H. J. 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. *Soil Biology & Biochemistry,* 40**,** 1352-1363.

STRASBURGER, E., KADEREIT, J. W., KÖRNER, C., KOST, B. & SONNEWALD, U. 2014. *Strasburger − Lehrbuch der Pflanzenwissenschaften*, Springer Berlin Heidelberg.

STRICKLAND, M. S., LEGGETT, Z. H., SUCRE, E. B. & BRADFORD, M. A. 2015. Biofuel intercropping effects on soil carbon and microbial activity. *Ecological Applications,* 25**,** 140-150.

SULLIVAN, D. M., NARTEA, T. J., BARY, A. I., COGGER, C. G. & MYHRE, E. A. 2004. Nitrogen availability and decomposition of urban yard trimmings in soil. *Soil Science,* 169**,** 697-707.

TALBOT, J. M., YELLE, D. J., NOWICK, J. & TRESEDER, K. K. 2012. Litter decay rates are determined by lignin chemistry. *Biogeochemistry,* 108**,** 279-295.

TAO, G., LESTANDER, T. A., GELADI, P. & XIONG, S. 2012. Biomass properties in association with plant species and assortments I: A synthesis based on literature data of energy properties. *Renewable & Sustainable Energy Reviews,* 16**,** 3481-3506.

THOMAS, S. C. & MARTIN, A. R. 2012. Carbon Content of Tree Tissues: A Synthesis. *Forests,* 3**,** 332-352.

THOMSEN, I. K., OLESEN, J. E., MOLLER, H. B., SORENSEN, P. & CHRISTENSEN, B. T. 2013. Carbon dynamics and retention in soil after anaerobic digestion of dairy cattle feed and faeces. *Soil Biology & Biochemistry,* 58**,** 82-87.

THURIES, L., PANSU, M., LARRE-LARROUY, M. C. & FELLER, C. 2002. Biochemical composition and mineralization kinetics of organic inputs in a sandy soil. *Soil Biology & Biochemistry,* 34**,** 239-250.

TORRES-CLIMENT, A., MARTIN-MATA, J., MARHUENDA-EGEA, F., MORAL, R., BARBER, X., PEREZ-MURCIA, M. D. & PAREDES, C. 2015. Composting of the Solid Phase of Digestate from Biogas Production: Optimization of the Moisture, C/N Ratio, and pH Conditions. *Communications in Soil Science and Plant Analysis,* 46**,** 197-207.

TRESEDER, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist,* 164**,** 347-355.

TRESEDER, K. K. 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters,* 11**,** 1111-1120.

TRINSOUTROT, I., RECOUS, S., BENTZ, B., LINERES, M., CHENEBY, D. & NICOLARDOT, B. 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. *Soil Science Society of America Journal,* 64**,** 918-926.

USDA. 2015. *Soil texture calculator* [Online]. United States Department of Agriculture. Available: http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/?cid=nrcs142p2_054167 [Accessed 2015].

USMAN, A. R. A., ABDULJABBAR, A., VITHANAGE, M., OK, Y. S., AHMAD, M., AHMAD, M., ELFAKI, J., ABDULAZEEM, S. S. & AL-WABEL, M. I. 2015. Biochar production from date palm waste: Charring temperature induced changes in composition and surface chemistry. *Journal of Analytical and Applied Pyrolysis,* 115**,** 392-400.

VAN SOEST, P. J., ROBERTSON, J. B. & LEWIS, B. A. 1991. METHODS FOR DIETARY FIBER NEUTRAL DETERGENT FIBER AND NONSTARCH POLYSACCHARIDES IN RELATION TO ANIMAL NUTRITION. *Journal of Dairy Science,* 74**,** 3583-3597.

VAN SOEST, P. J. & WINE, R. H. 1967. Use of detergents in the analysis of fibrous feeds: IV. Determination of plant cell-wall constituents. *Ass Offic Anal Chem J,* 50**,** 50-55.

VDLUFA 1976. *VDLUFA Methodenbuch,* Darmstadt, VDLUFA-Verlag.

VERLOOP, J., HILHORST, G. J., PRONK, A. A., SEBEK, L. B., VAN KEULEN, H., JANSSEN, B. H. & VAN ITTERSUM, M. K. 2015. Organic matter dynamics in an intensive dairy production system on a Dutch Spodosol. *Geoderma,* 237**,** 159-167.

VITOUSEK, P. M., ABER, J. D., HOWARTH, R. W., LIKENS, G. E., MATSON, P. A., SCHINDLER, D. W., SCHLESINGER, W. H. & TILMAN, D. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications,* 7**,** 737-750.

VOISIN, A.-S., GUEGUEN, J., HUYGHE, C., JEUFFROY, M.-H., MAGRINI, M.-B., MEYNARD, J.-M., MOUGEL, C., PELLERIN, S. & PELZER, E. 2014. Legumes for feed, food, biomaterials and bioenergy in Europe: a review. *Agronomy for Sustainable Development,* 34**,** 361-380.

VON LENGERKEN, J. & ZIMMERMANN, K. 1991. *Handbuch Futtermittelprüfung,* Berlin, Deutscher Landwirtschaftsverlag.

WAGAI, R., KISHIMOTO-MO, A. W., YONEMURA, S., SHIRATO, Y., HIRADATE, S. & YAGASAKI, Y. 2013. Linking temperature sensitivity of soil organic matter decomposition to its molecular structure, accessibility, and microbial physiology. *Global Change Biology,* 19**,** 1114-1125.

WALDROP, M. P. & ZAK, D. R. 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. *Ecosystems,* 9**,** 921-933.

WANG, W. J., BALDOCKA, J. A., DALALA, R. C. & MOODY, P. W. 2004. Decomposition dynamics of plant materials in relation to nitrogen availability and biochemistry determined by NMR and wet-chemical analysis. *Soil Biology & Biochemistry,* 36**,** 2045-2058.

WATTS, C. W., WHALLEY, W. R., BROOKES, P. C., DEVONSHIRE, B. J. & WHITMORE, A. P. 2005. Biological and physical processes that mediate micro-aggregation of clays. *Soil Science,* 170**,** 573-583.

WATTS, C. W., WHALLEY, W. R., LONGSTAFF, D., WHITE, R. P., BROOKE, P. C. & WHITMORE, A. P. 2001. Aggregation of a soil with different cropping histories following the addition of organic materials. *Soil Use and Management,* 17**,** 263-268.

WESSEN, B. & BERG, B. 1986. LONG-TERM DECOMPOSITION OF BARLEY STRAW - CHEMICAL-CHANGES AND INGROWTH OF FUNGAL MYCELIUM. *Soil Biology & Biochemistry,* 18**,** 53-59.

WILHELM, W. W., JOHNSON, J. M. F., HATFIELD, J. L., VOORHEES, W. B. & LINDEN, D. R. 2004. Crop and soil productivity response to corn residue removal: A literature review. *Agronomy Journal,* 96**,** 1-17.

WINKELMANN, W., ELLMER, F. & ZEITZ, J. 2006. Influence of differentiated organic-mineral fertilization on fractions of particulate soil organic matter. *Archives of Agronomy and Soil Science,* 52**,** 365-376.

WOODWELL, G. M. & WHITTAKER, R. H. 1968. PRIMARY PRODUCTION IN TERRESTRIAL ECOSYSTEMS. *American Zoologist,* 8**,** 19-&.

YEMM, E. W. & WILLIS, A. J. 1954. THE ESTIMATION OF CARBOHYDRATES IN PLANT EXTRACTS BY ANTHRONE. *Biochemical Journal,* 57**,** 508-514.

ZENG, D. H., MAO, R., CHANG, S. X., LI, L. J. & YANG, D. 2010. Carbon mineralization of tree leaf litter and crop residues from poplar-based agroforestry systems in Northeast China: A laboratory study. *Applied Soil Ecology,* 44**,** 133-137.

ZHANG, P., TIAN, X. J., HE, X. B., SONG, F. Q., REN, L. L. & JIANG, P. 2008. Effect of litter quality on its decomposition in broadleaf and coniferous forest. *European Journal of Soil Biology,* 44**,** 392-399.

ZHOU, G. Y., LIU, S. G., LI, Z., ZHANG, D. Q., TANG, X. L., ZHOU, C. Y., YAN, J. H. & MO, J. M. 2006. Old-growth forests can accumulate carbon in soils. *Science,* 314**,** 1417-1417.

Acknowledgements

I like to thank **Prof. Dr. Christof Engels** for enabling me to write this thesis and to develop this topic systematically in the versatile and challenging field of decomposition. I am very grateful to him for always being available as a guide, open-minded for personal specifications, and yet clearly communicating requirements and standards. I especially like to thank him for the complex nature of his support, setting me into the position to take the next steps.

Many thanks go to **Prof. Dr. Paolo Nannipieri** and **Prof. Dr. Eckhard George**, for acting as co-referees of this thesis.

The financial support of the **German federal Agency for Agriculture and Food** (project 22401112) is gratefully acknowledged.

Special thanks go to **Michael Baumecker** and **Jörg Schmidt** for performing the field experiment in Berlin Dahlem and supporting me with special access to all the inventory of the experimental station and of course the IOSDV long-term field experiment.

I am deeply indebted to **Carmen Wolfram-Wienberg** for all her assistance on the field, in the lab, and in the greenhouse throughout the entire duration of experimental work and even beyond this time.

I want to say thank you to **Monika Heller and the team of our division "Plant nutrition and fertilisation"** for all the support on the field and in the lab, conducting thousands of manual titrations.

I thank **Dr. Kirsten Weiss and the team of the central analytical laboratory** for all the support in biochemical analyses of soils and EOC.

Special thanks also go to **Dr. Kathlin Schweitzer** for always being on hand, when soil scientific questions arose.

Special thank also goes to **Dr. Bärbel Kroschewski and the division of biometry**, for all advice and support in statistical issues.

After all, the people who made the time of my PhD an awesome one, were the colleagues at the Faculty of Life Sciences and associated institutions in our research project, some of which I like to mention here: **Dr. Annika Nerlich, Holger Beßler, Sven Höcker, Lucas Knebl, Dr. Ralph Gründling, Dr. Uwe Franko, Dr. Christopher Brock, Anna Adam, Marissa Bieber, Martin Miersch, and Theo Radelhof**.

Last but not least, I want to express my deep gratitude and love to my family, most of all **Ilona Rahel Mewes, Oliver Paul Mewes, Gabriele and Paul-Rainer Mewes, Hedi Szabó and Reto De Pizzol, Wiebke Mewes, Marie Mewes, Siegfried and Marianne Dietsch, Erna Mewes, and in memorial Paul Mewes** for their unconditional love and support, bringing me to life and agriculture.